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(54) Title: PROTEIN-COUPLED RECEPTOR

(57) Abstract: The present application provides a gene encoding a G protein-coupled receptor termed nGPCR-14; constructs and recombinant host cells incorporating the genes; the nGPCR-14 polypeptides encoded by the gene; antibodies to the nGPCR-x polypeptides; and methods of making and using all of the foregoing.

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PROTEIN-COUPLED RECEPTOR

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FIELD OF THE INVENTION

The present invention relates generally to the fields of genetics and cellular and molecular biology. More particularly, the invention relates to novel G protein coupled receptors, to polynucleotides that encode such novel receptors, to reagents such as antibodies, probes, primers and kits comprising such antibodies, probes, primers related to the same, and to methods which use the novel G protein coupled receptors, polynucleotides or reagents.

BACKGROUND OF THE INVENTION

The G protein-coupled receptors (GPCRs) form a vast superfamily of cell surface receptors which are characterized by an amino-terminal extracellular domain, a carboxyl-terminal intracellular domain, and a serpentine structure that passes through the cell membrane seven times. Hence, such receptors are sometimes also referred to as seven transmembrane (7TM) receptors. These seven transmembrane domains define three extracellular loops and three intracellular loops, in addition to the amino- and carboxy-terminal domains. The extracellular portions of the receptor have a role in recognizing and binding one or more extracellular binding partners (*e.g.*, ligands), whereas the intracellular portions have a role in recognizing and communicating with downstream molecules in the signal transduction cascade.

The G protein-coupled receptors bind a variety of ligands including calcium ions, hormones, chemokines, neuropeptides, neurotransmitters, nucleotides, lipids, odorants, and even photons, and are important in the normal (and sometimes the aberrant) function of many cell types. [See generally Strosberg, *Eur. J. Biochem.* 196:1-10 (1991) and Bohm *et al.*, *Biochem J.* 322:1-18 (1997).] When a specific ligand binds to its corresponding receptor, the

ligand typically stimulates the receptor to activate a specific heterotrimeric guanine-nucleotide-binding regulatory protein (G-protein) that is coupled to the intracellular portion of the receptor. The G protein in turn transmits a signal to an effector molecule within the cell, by either stimulating or inhibiting the activity of that effector molecule.

5 These effector molecules include adenylate cyclase, phospholipases and ion channels. Adenylate cyclase and phospholipases are enzymes that are involved in the production of the second messenger molecules cAMP, inositol triphosphate and diacylglycerol. It is through this sequence of events that an extracellular ligand stimuli exerts intracellular changes through a G protein-coupled receptor. Each such receptor has its own characteristic primary

10 structure, expression pattern, ligand-binding profile, and intracellular effector system.

Because of the vital role of G protein-coupled receptors in the communication between cells and their environment, such receptors are attractive targets for therapeutic intervention, for example by activating or antagonizing such receptors. For receptors having a known ligand, the identification of agonists or antagonists may be sought specifically to

15 enhance or inhibit the action of the ligand. Some G protein-coupled receptors have roles in disease pathogenesis (*e.g.*, certain chemokine receptors that act as HIV co-receptors may have a role in AIDS pathogenesis), and are attractive targets for therapeutic intervention even in the absence of knowledge of the natural ligand of the receptor. Other receptors are attractive targets for therapeutic intervention by virtue of their expression pattern in tissues or

20 cell types that are themselves attractive targets for therapeutic intervention. Examples of this latter category of receptors include receptors expressed in immune cells, which can be targeted to either inhibit autoimmune responses or to enhance immune responses to fight pathogens or cancer; and receptors expressed in the brain or other neural organs and tissues, which are likely targets in the treatment of schizophrenia, depression, bipolar disease, or

25 other neurological disorders. This latter category of receptor is also useful as a marker for identifying and/or purifying (*e.g.*, via fluorescence-activated cell sorting) cellular subtypes that express the receptor. Unfortunately, only a limited number of G protein receptors from the central nervous system (CNS) are known. Thus, a need exists for G protein-coupled receptors that have been identified and show promise as targets for therapeutic intervention in

30 a variety of animals, including humans.

SUMMARY OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule that comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence

homologous to even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192, or a fragment thereof. The nucleic acid molecule encodes at least a portion of nGPCR-x. In some embodiments, the nucleic acid molecule comprises a sequence that encodes a polypeptide comprising even numbered sequences
5 ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192, or a fragment thereof. In some embodiments, the nucleic acid molecule comprises a sequence homologous to odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, or a fragment thereof. In some embodiments, the nucleic acid molecule comprises a sequence selected from the group consisting of odd
10 numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, and fragments thereof.

According to some embodiments, the present invention provides vectors which comprise the nucleic acid molecule of the invention. In some embodiments, the vector is an expression vector.

15 According to some embodiments, the present invention provides host cells which comprise the vectors of the invention. In some embodiments, the host cells comprise expression vectors.

The present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to at least a portion of a sequence from an odd numbered
20 sequence ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, said portion comprising at least 10 nucleotides.

The present invention provides a method of producing a polypeptide comprising a sequence from an even numbered sequence ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192, or a homolog or fragment thereof. The method
25 comprising the steps of introducing a recombinant expression vector that includes a nucleotide sequence that encodes the polypeptide into a compatible host cell, growing the host cell under conditions for expression of the polypeptide and recovering the polypeptide.

The present invention provides an isolated antibody which binds to an epitope on a polypeptide comprising a sequence from an even numbered sequence ranging from SEQ ID
30 NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192, or a homolog or fragment thereof.

The present invention provides a method of inducing an immune response in a mammal against a polypeptide comprising a sequence from an even numbered sequence ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192, or a

homolog or fragment thereof. The method comprises administering to a mammal an amount of the polypeptide sufficient to induce said immune response.

The present invention provides a method for identifying a compound which binds nGPCR-x. The method comprises the steps of: contacting nGPCR-x with a compound and
5 determining whether the compound binds nGPCR-x.

The present invention provides a method for identifying a compound which binds a nucleic acid molecule encoding nGPCR-x. The method comprises the steps of contacting said nucleic acid molecule encoding nGPCR-x with a compound and determining whether said compound binds said nucleic acid molecule.

10 The present invention provides a method for identifying a compound which modulates the activity of nGPCR-x. The method comprises the steps of contacting nGPCR-x with a compound and determining whether nGPCR-x activity has been modulated.

The present invention provides a method of identifying an animal homolog of nGPCR-x. The method comprises the steps screening a nucleic acid database of the animal
15 with an odd numbered sequence ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, or a portion thereof and determining whether a portion of said library or database is homologous to said odd numbered sequence ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, or portion thereof.

The present invention provides a method of identifying an animal homolog of
20 nGPCR-x. The methods comprises the steps screening a nucleic acid library of the animal with a nucleic acid molecule having an odd numbered nucleotide sequence ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191, or a portion thereof; and determining whether a portion of said library or database is homologous to said odd numbered nucleotide sequence ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO:
25 185 and SEQ ID NO: 191, or a portion thereof.

Another aspect of the present invention relates to methods of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor. The methods comprise the steps of assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression, or
30 biological activity of at least one nGPCR that is expressed in the brain. The nGPCR comprise an amino acid sequence selected from the group consisting of: SEQ ID NO:74, SEQ ID NO:186, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:90, and SEQ ID NO:94, and allelic variants thereof. A diagnosis of the disorder or predisposition is made from the presence or absence of the mutation. The

presence of a mutation altering the amino acid sequence, expression, or biological activity of the nGPCR in the nucleic acid correlates with an increased risk of developing the disorder.

The present invention further relates to methods of screening for a nGPCR-40 or nGPCR-54 hereditary schizophrenia genotype in a human patient. The methods comprise the steps of providing a biological sample comprising nucleic acid from the patient, in which the nucleic acid includes sequences corresponding to alleles of nGPCR-40 or nGPCR-54. The presence of one or more mutations in the nGPCR-40 allele or the nGPCR-54 allele is detected indicative of a hereditary schizophrenia genotype.

The present invention provides kits for screening a human subject to diagnose schizophrenia or a genetic predisposition therefor. The kits include an oligonucleotide useful as a probe for identifying polymorphisms in a human nGPCR-40 gene or a human nGPCR-54 gene. The oligonucleotide comprises 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human nGPCR-40 or nGPCR-54 gene sequence or nGPCR-40 or nGPCR-54 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution. The kit also includes a media packaged with the oligonucleotide. The media contains information for identifying polymorphisms that correlate with schizophrenia or a genetic predisposition therefor, the polymorphisms being identifiable using the oligonucleotide as a probe.

The present invention further relates to methods of identifying nGPCR allelic variants that correlates with mental disorders. The methods comprise the steps of providing biological samples that comprise nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny, and detecting in the nucleic acid the presence of one or more mutations in an nGPCR that is expressed in the brain. The nGPCR comprises an amino acid sequence selected from the group consisting of SEQ ID NO:74, SEQ ID NO:186, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:90, and SEQ ID NO:94, and allelic variants thereof. The nucleic acid includes sequences corresponding to the gene or genes encoding nGPCR. The one or more mutations detected indicate an allelic variant that correlates with a mental disorder.

The present invention further relates to purified polynucleotides comprising nucleotide sequences encoding alleles of nGPCR-40 or nGPCR-54 from a human with schizophrenia. The polynucleotide hybridizes to the complement of SEQ ID NO:83 or of SEQ ID NO:85 under the following hybridization conditions: (a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10%

dextran sulfate and (b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS. The polynucleotide that encodes nGPCR-40 or nGPCR-54 amino acid sequence of the human differs from SEQ ID NO:84 or SEQ ID NO:86 by at least one residue.

5 The present invention also provides methods for identifying a modulator of biological activity of nGPCR-40 or nGPCR-54 comprising the steps of contacting a cell that expresses nGPCR-40 or nGPCR-54 in the presence and in the absence of a putative modulator compound and measuring nGPCR-40 or nGPCR-54 biological activity in the cell. The decreased or increased nGPCR-40 or nGPCR-54 biological activity in the presence versus
10 absence of the putative modulator is indicative of a modulator of biological activity.

 The present invention further provides methods to identify compounds useful for the treatment of schizophrenia. The methods comprise the steps of contacting a composition comprising nGPCR-40 with a compound suspected of binding nGPCR-40 or contacting a composition comprising nGPCR-54 with a compound suspected of binding nGPCR-54. The
15 binding between nGPCR-40 and the compound suspected of binding nGPCR-40 or between nGPCR-54 and the compound suspected of binding nGPCR-54 is detected. Compounds identified as binding nGPCR-40 or nGPCR-54 are candidate compounds useful for the treatment of schizophrenia.

 The present invention further provides methods for identifying a compound useful as
20 a modulator of binding between nGPCR-40 and a binding partner of nGPCR-40 or between nGPCR-54 and a binding partner of nGPCR-54. The methods comprise the steps of contacting the binding partner and a composition comprising nGPCR-40 or nGPCR-54 in the presence and in the absence of a putative modulator compound and detecting binding between the binding partner and nGPCR-40 or nGPCR-54. Decreased or increased binding
25 between the binding partner and nGPCR-40 or nGPCR-54 in the presence of the putative modulator, as compared to binding in the absence of the putative modulator is indicative a modulator compound useful for the treatment of schizophrenia.

 Another aspect of the present invention relates to methods of purifying a G protein from a sample containing a G protein. The methods comprise the steps of contacting the
30 sample with an nGPCR for a time sufficient to allow the G protein to form a complex with the nGPCR; isolating the complex from remaining components of the sample; maintaining the complex under conditions which result in dissociation of the G protein from the nGPCR; and isolating said G protein from the nGPCR.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

Various definitions are made throughout this document. Most words have the meaning that would be attributed to those words by one skilled in the art. Words specifically
5 defined either below or elsewhere in this document have the meaning provided in the context of the present invention as a whole and as are typically understood by those skilled in the art.

"Synthesized" as used herein and understood in the art, refers to polynucleotides produced by purely chemical, as opposed to enzymatic, methods. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially"
10 synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means.

By the term "region" is meant a physically contiguous portion of the primary structure of a biomolecule. In the case of proteins, a region is defined by a contiguous portion of the amino acid sequence of that protein.

15 The term "domain" is herein defined as referring to a structural part of a biomolecule that contributes to a known or suspected function of the biomolecule. Domains may be co-extensive with regions or portions thereof; domains may also incorporate a portion of a biomolecule that is distinct from a particular region, in addition to all or part of that region. Examples of GPCR protein domains include, but are not limited to, the extracellular (*i.e.*, N-
20 terminal), transmembrane and cytoplasmic (*i.e.*, C-terminal) domains, which are co-extensive with like-named regions of GPCRs; each of the seven transmembrane segments of a GPCR; and each of the loop segments (both extracellular and intracellular loops) connecting adjacent transmembrane segments.

As used herein, the term "activity" refers to a variety of measurable indicia suggesting
25 or revealing binding, either direct or indirect; affecting a response, *i.e.* having a measurable affect in response to some exposure or stimulus, including, for example, the affinity of a compound for directly binding a polypeptide or polynucleotide of the invention, or, for example, measurement of amounts of upstream or downstream proteins or other similar functions after some stimulus or event.

30 Unless indicated otherwise, as used herein, the abbreviation in lower case (gpcr) refers to a gene, cDNA, RNA or nucleic acid sequence, while the upper case version (GPCR) refers to a protein, polypeptide, peptide, oligopeptide, or amino acid sequence. The term

“nGPCR-x” refers to any of the nGPCRs taught herein, while specific reference to a nGPCR (for example nGPCR-5) refers only to that specific nGPCR.

As used herein, the term “antibody” is meant to refer to complete, intact antibodies, and Fab, Fab’, F(ab)2, and other fragments thereof. Complete, intact antibodies include
5 monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies.

As used herein, the term “binding” means the physical or chemical interaction between two proteins or compounds or associated proteins or compounds or combinations thereof. Binding includes ionic, non-ionic, Hydrogen bonds, Van der Waals, hydrophobic
10 interactions, etc. The physical interaction, the binding, can be either direct or indirect, indirect being through or due to the effects of another protein or compound. Direct binding refers to interactions that do not take place through or due to the effect of another protein or compound but instead are without other substantial chemical intermediates. Binding may be detected in many different manners. As a non-limiting example, the physical binding
15 interaction between a nGPCR-x of the invention and a compound can be detected using a labeled compound. Alternatively, functional evidence of binding can be detected using, for example, a cell transfected with and expressing a nGPCR-x of the invention. Binding of the transfected cell to a ligand of the nGPCR that was transfected into the cell provides functional evidence of binding. Other methods of detecting binding are well-known to those
20 of skill in the art.

As used herein, the term “compound” means any identifiable chemical or molecule, including, but not limited to, small molecule, peptide, protein, sugar, nucleotide, or nucleic acid, and such compound can be natural or synthetic.

As used herein, the term “complementary” refers to Watson-Crick basepairing
25 between nucleotide units of a nucleic acid molecule.

As used herein, the term “contacting” means bringing together, either directly or indirectly, a compound into physical proximity to a polypeptide or polynucleotide of the invention. The polypeptide or polynucleotide can be in any number of buffers, salts, solutions etc. Contacting includes, for example, placing the compound into a beaker,
30 microtiter plate, cell culture flask, or a microarray, such as a gene chip, or the like, which contains the nucleic acid molecule, or polypeptide encoding the nGPCR or fragment thereof.

As used herein, the phrase “homologous nucleotide sequence,” or “homologous amino acid sequence,” or variations thereof, refers to sequences characterized by a homology, at the nucleotide level or amino acid level, of at least the specified percentage. Homologous

nucleotide sequences include those sequences coding for isoforms of proteins. Such isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences include nucleotide sequences encoding for a protein of a species other than humans, including, but not limited to, mammals. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding other known GPCRs. Homologous amino acid sequences include those amino acid sequences which contain conservative amino acid substitutions and which polypeptides have the same binding and/or activity. A homologous amino acid sequence does not, however, include the amino acid sequence encoding other known GPCRs. Percent homology can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489, which is incorporated herein by reference in its entirety).

As used herein, the term "isolated" nucleic acid molecule refers to a nucleic acid molecule (DNA or RNA) that has been removed from its native environment. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules.

As used herein, the terms "modulates" or "modifies" means an increase or decrease in the amount, quality, or effect of a particular activity or protein.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues which has a sufficient number of bases to be used in a polymerase chain reaction (PCR). This short sequence is based on (or designed from) a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

As used herein, the term "probe" refers to nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides, depending on

use. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. They may be single- or double-stranded and carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

The term "preventing" refers to decreasing the probability that an organism contracts or develops an abnormal condition.

The term "treating" refers to having a therapeutic effect and at least partially alleviating or abrogating an abnormal condition in the organism.

The term "therapeutic effect" refers to the inhibition or activation factors causing or contributing to the abnormal condition. A therapeutic effect relieves to some extent one or more of the symptoms of the abnormal condition. In reference to the treatment of abnormal conditions, a therapeutic effect can refer to one or more of the following: (a) an increase in the proliferation, growth, and/or differentiation of cells; (b) inhibition (*i.e.*, slowing or stopping) of cell death; (c) inhibition of degeneration; (d) relieving to some extent one or more of the symptoms associated with the abnormal condition; and (e) enhancing the function of the affected population of cells. Compounds demonstrating efficacy against abnormal conditions can be identified as described herein.

The term "abnormal condition" refers to a function in the cells or tissues of an organism that deviates from their normal functions in that organism. An abnormal condition can relate to cell proliferation, cell differentiation, cell signaling, or cell survival. An abnormal condition may also include obesity, diabetic complications such as retinal degeneration, and irregularities in glucose uptake and metabolism, and fatty acid uptake and metabolism.

Abnormal cell proliferative conditions include cancers such as fibrotic and mesangial disorders, abnormal angiogenesis and vasculogenesis, wound healing, psoriasis, diabetes mellitus, and inflammation.

Abnormal differentiation conditions include, but are not limited to, neurodegenerative disorders, slow wound healing rates, and slow tissue grafting healing rates. Abnormal cell signaling conditions include, but are not limited to, psychiatric disorders involving excess neurotransmitter activity.

Abnormal cell survival conditions may also relate to conditions in which programmed cell death (apoptosis) pathways are activated or abrogated. A number of protein kinases are

associated with the apoptosis pathways. Aberrations in the function of any one of the protein kinases could lead to cell immortality or premature cell death.

The term "administering" relates to a method of incorporating a compound into cells or tissues of an organism. The abnormal condition can be prevented or treated when the cells or tissues of the organism exist within the organism or outside of the organism. Cells existing outside the organism can be maintained or grown in cell culture dishes. For cells harbored within the organism, many techniques exist in the art to administer compounds, including (but not limited to) oral, parenteral, dermal, injection, and aerosol applications. For cells outside of the organism, multiple techniques exist in the art to administer the compounds, including (but not limited to) cell microinjection techniques, transformation techniques and carrier techniques.

The abnormal condition can also be prevented or treated by administering a compound to a group of cells having an aberration in a signal transduction pathway to an organism. The effect of administering a compound on organism function can then be monitored. The organism is preferably a mouse, rat, rabbit, guinea pig or goat, more preferably a monkey or ape, and most preferably a human.

By "amplification" it is meant increased numbers of DNA or RNA in a cell compared with normal cells. "Amplification" as it refers to RNA can be the detectable presence of RNA in cells, since in some normal cells there is no basal expression of RNA. In other normal cells, a basal level of expression exists, therefore in these cases amplification is the detection of at least 1 to 2-fold, and preferably more, compared to the basal level.

As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a probe, primer, or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present in excess, at T_m , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (e.g. 10 to 50 nucleotides)

and at least about 60°C for longer probes, primers or oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

The amino acid sequences are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. The nucleotide sequences are presented by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission or (for amino acids) by three letters code.

Polynucleotides

The present invention provides purified and isolated polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands, both single- and double-stranded, including splice variants thereof) that encode unknown G protein-coupled receptors heretofore termed novel GPCRs, or nGPCRs. These genes are described herein and designated herein collectively as nGPCR-x (where x is 1, 3, 4, 5, 9, 11, 12, 14, 15, 18, 16, 17, 20, 21, 22, 24, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 40, 41, 53, 54, 55, 56, 57, 58, 59, or 60). That is, these genes are described herein and designated herein as nGPCR-1 (also referred to as beGPCR-1), nGPCR-3 (also referred to as beGPCR-3), nGPCR-4 (also referred to as beGPCR-4), nGPCR-5 (also referred to as beGPCR-5 and TL-GPCR-5), nGPCR-9 (also referred to as beGPCR-9), nGPCR-11 (also referred to as beGPCR-11), nGPCR-12 (also referred to as beGPCR-12), nGPCR-14 (also referred to as beGPCR-14), nGPCR-15 (also referred to as beGPCR-15), nGPCR-18 (also referred to as beGPCR-18), nGPCR-16 (also referred to as beGPCR-16), nGPCR-17 (also referred to as beGPCR-17), nGPCR-20 (also referred to as beGPCR-20), nGPCR-21 (also referred to as beGPCR-21), nGPCR-22 (also referred to as beGPCR-22), nGPCR-24 (also referred to as beGPCR-24), nGPCR-27 (also referred to as beGPCR-27), nGPCR-28 (also referred to as beGPCR-28), nGPCR-31 (also referred to as beGPCR-31), nGPCR-32 (also referred to as beGPCR-32), nGPCR-33 (also referred to as beGPCR-33), nGPCR-34 (also referred to as beGPCR-34), nGPCR-35 (also referred to as beGPCR-35), nGPCR-36 (also referred to as beGPCR-36), nGPCR-37 (also referred to as beGPCR-37), nGPCR-38 (also referred to as beGPCR-38), nGPCR-40 (also referred to as beGPCR-40), nGPCR-41 (also referred to as beGPCR-41), nGPCR-53, nGPCR-54, nGPCR-55, nGPCR-56, nGPCR-57, nGPCR-58, nGPCR-59, and nGPCR-60. Table 1 below identifies the novel gene sequence nGPCR-x designation, the SEQ ID NO: of the gene sequence, the SEQ ID NO: of the polypeptide

encoded thereby, and the U.S. Provisional Application in which the gene sequence has been disclosed.

Table 1

| nGPCR | Nucleotide Sequence (SEQ ID NO:) | Amino acid Sequence (SEQ ID NO:) | Originally filed in: | nGPCR | Nucleotide Sequence (SEQ ID NO:) | Amino acid Sequence (SEQ ID NO:) | Originally filed in: |
|-------|---|---|-------------------------|-------|---|---|-------------------------|
| 1 | 1 | 2 | A | 32 | 39 | 40 | B |
| 1 | 73 | 74 | E | 33 | 41 | 42 | C |
| 3 | 3 | 4 | A | 34 | 43 | 44 | C |
| 3 | 185 | 186 | P | 35 | 45 | 46 | C |
| 4 | 5 | 6 | A | 36 | 47 | 48 | C |
| 5 | 7 | 8 | A | 37 | 49 | 50 | C |
| 5 | 75 | 76 | F | 38 | 51 | 52 | C |
| 9 | 9 | 10 | A | 40 | 53 | 54 | C |
| 9 | 77 | 78 | G | 40 | 83 | 84 | J |
| 11 | 11 | 12 | A | 41 | 55 | 56 | C |
| 11 | 79 | 80 | H | 53 | 57 | 58 | D |
| 12 | 13 | 14 | A | 54 | 59 | 60 | D |
| 14 | 15 | 16 | A | 54 | 85 | 86 | K |
| 14 | 191 | 192 | herein | | | | |
| 15 | 17 | 18 | A | 55 | 61 | 62 | D |
| 18 | 19 | 20 | A | 56 | 63 | 64 | D |
| 16 | 21 | 22 | B | 56 | 87 | 88 | L |
| 16 | 81 | 82 | I | 56 | 89 | 90 | M |
| 17 | 23 | 24 | B | 57 | 65 | 66 | D |
| 20 | 25 | 26 | B | 58 | 67 | 68 | D |
| 21 | 27 | 28 | B | 58 | 91 | 92 | N |
| 22 | 29 | 30 | B | 58 | 93 | 94 | O |
| 24 | 31 | 32 | B | 59 | 69 | 70 | D |
| 27 | 33 | 34 | B | 60 | 71 | 72 | D |
| 28 | 35 | 36 | B | | | | |
| 31 | 37 | 38 | B | | | | |

Legend

A= Ser. No. 60/165,838
 B= Ser. No. 60/166,071
 C= Ser. No. 60/166,678
 D= Ser. No. 60/173,396
 E= Ser. No. 60/184,129
 F= Ser. No. 60/188,114
 G= Ser. No. 60/185,421
 H= Ser. No. 60/186,811

I= Ser. No. 60/186,530
 J= Ser. No. 60/207,094
 K= Ser. No. 60/203,111
 L= Ser. No. 60/190,310
 M= Ser. No. 60/201,190
 N= Ser. No. 60/185,554
 O= Ser. No. 60/190,800
 P= Ser. No. 60/198,568

When a specific nGPCR is identified (for example nGPCR-5), it is understood that only that specific nGPCR is being referred to.

As described in Example 4 below, the genes encoding nGPCR-1 (nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 73, amino acid sequence SEQ ID NO: 2, SEQ ID NO:74), nGPCR-9 (nucleic acid sequence SEQ ID NO:9, SEQ ID NO:77, amino acid sequence SEQ ID NO:10, SEQ ID NO:78), nGPCR-11 (nucleic acid sequence SEQ ID NO:11, SEQ ID NO:79, amino acid sequence SEQ ID NO:12, SEQ ID NO:80), nGPCR-16 (nucleic acid sequence SEQ ID NO: 21, SEQ ID NO:81, amino acid sequence SEQ ID NO:

22, SEQ ID NO:82), nGPCR-40 (nucleic acid sequence SEQ ID NO:53, SEQ ID NO:83, amino acid sequence SEQ ID NO:54, SEQ ID NO:84), nGPCR-54 (nucleic acid sequence SEQ ID NO:59, SEQ ID NO:85, amino acid sequence SEQ ID NO:60, SEQ ID NO: 86), nGPCR-56 (nucleic acid sequence SEQ ID NO:63, SEQ ID NO:87, SEQ ID NO:89, amino acid sequence SEQ ID NO:64, SEQ ID NO: 88, SEQ ID NO:90), nGPCR-58 (nucleic acid sequence SEQ ID NO:67, SEQ ID NO:91, SEQ ID NO:93, amino acid sequence SEQ ID NO:68, SEQ ID NO: 92, SEQ ID NO:94) and nGPCR-3 (nucleic acid sequence SEQ ID NO:3, SEQ ID NO:185, amino acid sequence SEQ ID NO:4, SEQ ID NO: 186) have been detected in brain tissue indicating that these n-GPCR-x proteins are neuroreceptors.

10 The invention provides purified and isolated polynucleotides (*e.g.*, cDNA, genomic DNA, synthetic DNA, RNA, or combinations thereof, whether single- or double-stranded) that comprise a nucleotide sequence encoding the amino acid sequence of the polypeptides of the invention. Such polynucleotides are useful for recombinantly expressing the receptor and also for detecting expression of the receptor in cells (*e.g.*, using Northern hybridization and *in situ* hybridization assays). Such polynucleotides also are useful in the design of antisense and other molecules for the suppression of the expression of nGPCR-x in a cultured cell, a tissue, or an animal; for therapeutic purposes; or to provide a model for diseases or conditions characterized by aberrant nGPCR-x expression. Specifically excluded from the definition of polynucleotides of the invention are entire isolated, non-recombinant native chromosomes of host cells. A preferred polynucleotide has the sequence of the sequence set forth in odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO:191, which correspond to naturally occurring nGPCR-x sequences. It will be appreciated that numerous other polynucleotide sequences exist that also encode nGPCR-x having the sequence set forth in even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192, due to the well-known degeneracy of the universal genetic code.

20 The invention also provides a purified and isolated polynucleotide comprising a nucleotide sequence that encodes a mammalian polypeptide, wherein the polynucleotide hybridizes to a polynucleotide having the sequence set forth in odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185, and SEQ ID NO:191, or the non-coding strand complementary thereto, under the following hybridization conditions:

30 (a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate; and

(b) washing 2 times for 30 minutes each at 60°C in a wash solution comprising 0.1% SSC, 1% SDS. Polynucleotides that encode a human allelic variant are highly preferred.

The present invention relates to molecules which comprise the gene sequences that encode the nGPCRs; constructs and recombinant host cells incorporating the gene sequences; the novel GPCR polypeptides encoded by the gene sequences; antibodies to the polypeptides and homologs; kits employing the polynucleotides and polypeptides, and methods of making and using all of the foregoing. In addition, the present invention relates to homologs of the gene sequences and of the polypeptides and methods of making and using the same.

Genomic DNA of the invention comprises the protein-coding region for a polypeptide of the invention and is also intended to include allelic variants thereof. It is widely understood that, for many genes, genomic DNA is transcribed into RNA transcripts that undergo one or more splicing events wherein intron (*i.e.*, non-coding regions) of the transcripts are removed, or "spliced out." RNA transcripts that can be spliced by alternative mechanisms, and therefore be subject to removal of different RNA sequences but still encode a nGPCR-x polypeptide, are referred to in the art as splice variants which are embraced by the invention. Splice variants comprehended by the invention therefore are encoded by the same original genomic DNA sequences but arise from distinct mRNA transcripts. Allelic variants are modified forms of a wild-type gene sequence, the modification resulting from recombination during chromosomal segregation or exposure to conditions which give rise to genetic mutation. Allelic variants, like wild type genes, are naturally occurring sequences (as opposed to non-naturally occurring variants that arise from *in vitro* manipulation).

The invention also comprehends cDNA that is obtained through reverse transcription of an RNA polynucleotide encoding nGPCR-x (conventionally followed by second strand synthesis of a complementary strand to provide a double-stranded DNA).

Preferred DNA sequences encoding human nGPCR-x polypeptides are set out in odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191. A preferred DNA of the invention comprises a double stranded molecule along with the complementary molecule (the "non-coding strand" or "complement") having a sequence unambiguously deducible from the coding strand according to Watson-Crick base pairing rules for DNA. Also preferred are other polynucleotides encoding the nGPCR-x polypeptide of even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO: 192 which differ in sequence from the polynucleotides of odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185

and SEQ ID NO:192, by virtue of the well-known degeneracy of the universal nuclear genetic code.

In a preferred embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence which encodes a fragment of polypeptide comprising a sequence of SEQ ID NO: 192. The fragment of the polypeptide comprising a sequence of SEQ ID NO: 192 comprises at least one or more amino acid residues from one or more of the following regions of SEQ ID NO: 192: amino acid residues 1 to 42 of SEQ ID NO:192; amino acid residues 68 to 77 of SEQ ID NO:192; amino acid residues 185 to 197 of SEQ ID NO: 192; or amino acid residues 293 to 513 of SEQ ID NO: 192.

In a preferred embodiment, the isolated nucleic acid comprises a nucleotide sequence of SEQ ID NO: 191, and fragments thereof, that encode a polypeptide having a sequence of SEQ ID NO: 192, or fragments thereof. The fragment of the nucleotide sequence of SEQ ID NO: 191 comprises at least one or more nucleotides from one or more of the following regions of SEQ ID NO: 191: nucleotides 1 to 193 of SEQ ID NO: 191; nucleotides 612 to 644 of SEQ ID NO:191; nucleotides 697 to 706 of SEQ ID NO:191; nucleotides 1011 to 1049 of SEQ ID NO: 191; nucleotides 1051 to 1057 of SEQ ID NO:191; nucleotides 1090 to 1096 of SEQ ID NO:191; or nucleotides 1141 to 1642 of SEQ ID NO:191.

The invention further embraces other species, preferably mammalian, homologs of the human nGPCR-x DNA. Species homologs, sometimes referred to as "orthologs," in general, share at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with human DNA of the invention. Generally, percent sequence "homology" with respect to polynucleotides of the invention may be calculated as the percentage of nucleotide bases in the candidate sequence that are identical to nucleotides in the nGPCR-x sequence set forth in odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO:191, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Polynucleotides of the invention permit identification and isolation of polynucleotides encoding related nGPCR-x polypeptides, such as human allelic variants and species homologs, by well-known techniques including Southern and/or Northern hybridization, and polymerase chain reaction (PCR). Examples of related polynucleotides include human and non-human genomic sequences, including allelic variants, as well as polynucleotides encoding polypeptides homologous to nGPCR-x and structurally related polypeptides sharing

one or more biological, immunological, and/or physical properties of nGPCR-x. Non-human species genes encoding proteins homologous to nGPCR-x can also be identified by Southern and/or PCR analysis and are useful in animal models for nGPCR-x disorders. Knowledge of the sequence of a human nGPCR-x DNA also makes possible through use of Southern hybridization or polymerase chain reaction (PCR) the identification of genomic DNA sequences encoding nGPCR-x expression control regulatory sequences such as promoters, operators, enhancers, repressors, and the like. Polynucleotides of the invention are also useful in hybridization assays to detect the capacity of cells to express nGPCR-x. Polynucleotides of the invention may also provide a basis for diagnostic methods useful for identifying a genetic alteration(s) in a nGPCR-x locus that underlies a disease state or states, which information is useful both for diagnosis and for selection of therapeutic strategies.

According to the present invention, the nGPCR-x nucleotide sequences disclosed herein may be used to identify homologs of the nGPCR-x, in other animals, including but not limited to humans and other mammals, and invertebrates. Any of the nucleotide sequences disclosed herein, or any portion thereof, can be used, for example, as probes to screen databases or nucleic acid libraries, such as, for example, genomic or cDNA libraries, to identify homologs, using screening procedures well known to those skilled in the art. Accordingly, homologs having at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 100% homology with nGPCR-x sequences can be identified.

The disclosure herein of full-length polynucleotides encoding nGPCR-x polypeptides makes readily available to the worker of ordinary skill in the art every possible fragment of the full-length polynucleotide.

One preferred embodiment of the present invention provides an isolated nucleic acid molecule comprising a sequence homologous to odd numbered sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:93, SEQ ID NO: 185 and SEQ ID NO:191, and fragments thereof. Another preferred embodiment provides an isolated nucleic acid molecule comprising a sequence selected from the group of odd numbered sequences consisting of SEQ ID NO:1 to SEQ ID NO:93, SEQ ID NO:185 and SEQ ID NO:191, and fragments thereof.

As used in the present invention, fragments of nGPCR-x-encoding polynucleotides comprise at least 10, and preferably at least 12, 14, 16, 18, 20, 25, 50, or 75 consecutive nucleotides of a polynucleotide encoding nGPCR-x. Preferably, fragment polynucleotides of

the invention comprise sequences unique to the nGPCR-x-encoding polynucleotide sequence, and therefore hybridize under highly stringent or moderately stringent conditions only (*i.e.*, “specifically”) to polynucleotides encoding nGPCR-x (or fragments thereof). Polynucleotide fragments of genomic sequences of the invention comprise not only sequences unique to the coding region, but also include fragments of the full-length sequence derived from introns, regulatory regions, and/or other non-translated sequences. Sequences unique to polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, *e.g.*, those made available in public sequence databases. Such sequences also are recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labeled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labeling.

Fragment polynucleotides are particularly useful as probes for detection of full-length or fragments of nGPCR-x polynucleotides. One or more polynucleotides can be included in kits that are used to detect the presence of a polynucleotide encoding nGPCR-x, or used to detect variations in a polynucleotide sequence encoding nGPCR-x.

The invention also embraces DNAs encoding nGPCR-x polypeptides that hybridize under moderately stringent or high stringency conditions to the non-coding strand, or complement, of the polynucleotides set forth in odd numbered sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO:192.

Exemplary highly stringent hybridization conditions are as follows: hybridization at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel *et al.* (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, *et al.*, (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

With the knowledge of the nucleotide sequence information disclosed in the present invention, one skilled in the art can identify and obtain nucleotide sequences which encode nGPCR-x from different sources (*i.e.*, different tissues or different organisms) through a variety of means well known to the skilled artisan and as disclosed by, for example,
5 Sambrook et al., "Molecular cloning: a laboratory manual", Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), which is incorporated herein by reference in its entirety.

For example, DNA that encodes nGPCR-x may be obtained by screening of mRNA, cDNA, or genomic DNA with oligonucleotide probes generated from the nGPCR-x gene
10 sequence information provided herein. Probes may be labeled with a detectable group, such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with procedures known to the skilled artisan and used in conventional hybridization assays, as described by, for example, Sambrook *et al.*

A nucleic acid molecule comprising any of the nGPCR-x nucleotide sequences
15 described above can alternatively be synthesized by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers produced from the nucleotide sequences provided herein. See U.S. Patent Numbers 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis. The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been
20 previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

A wide variety of alternative cloning and *in vitro* amplification methodologies are
25 well known to those skilled in the art. Examples of these techniques are found in, for example, Berger *et al.*, *Guide to Molecular Cloning Techniques*, Methods in Enzymology 152, Academic Press, Inc., San Diego, CA (Berger), which is incorporated herein by reference in its entirety.

Automated sequencing methods can be used to obtain or verify the nucleotide
30 sequence of nGPCR-x. The nGPCR-x nucleotide sequences of the present invention are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule.

The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in a sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual
5 nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation.

The nucleic acid molecules of the present invention, and fragments derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain disorders, as well as for genetic mapping.

The polynucleotide sequence information provided by the invention makes possible
10 large-scale expression of the encoded polypeptide by techniques well known and routinely practiced in the art.

Vectors

Another aspect of the present invention is directed to vectors, or recombinant expression vectors, comprising any of the nucleic acid molecules described above. Vectors
15 are used herein either to amplify DNA or RNA encoding nGPCR-x and/or to express DNA which encodes nGPCR-x. Preferred vectors include, but are not limited to, plasmids, phages, cosmids, episomes, viral particles or viruses, and integratable DNA fragments (*i.e.*, fragments integratable into the host genome by homologous recombination). Preferred viral particles include, but are not limited to, adenoviruses, baculoviruses, parvoviruses, herpesviruses,
20 poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses. Preferred expression vectors include, but are not limited to, pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Other expression vectors include, but are not limited to, pSPORTTM vectors, pGEMTM vectors (Promega), pPROEXvectorsTM (LTI, Bethesda, MD), BluescriptTM vectors (Stratagene), pQETM vectors (Qiagen), pSE420TM
25 (Invitrogen), and pYES2TM(Invitrogen).

Expression constructs preferably comprise GPCR-x-encoding polynucleotides operatively linked to an endogenous or exogenous expression control DNA sequence and a transcription terminator. Expression control DNA sequences include promoters, enhancers, operators, and regulatory element binding sites generally, and are typically selected based on
30 the expression systems in which the expression construct is to be utilized. Preferred promoter and enhancer sequences are generally selected for the ability to increase gene expression, while operator sequences are generally selected for the ability to regulate gene expression. Expression constructs of the invention may also include sequences encoding one or more selectable markers that permit identification of host cells bearing the construct. Expression

constructs may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell. Preferred constructs of the invention also include sequences necessary for replication in a host cell.

Expression constructs are preferably utilized for production of an encoded protein, but
5 may also be utilized simply to amplify a nGPCR-x-encoding polynucleotide sequence. In preferred embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Autonomously replicating recombinant expression constructs such as plasmid and viral DNA vectors incorporating polynucleotides of the invention are also provided.

10 Preferred expression vectors are replicable DNA constructs in which a DNA sequence encoding nGPCR-x is operably linked or connected to suitable control sequences capable of effecting the expression of the nGPCR-x in a suitable host. DNA regions are operably linked or connected when they are functionally related to each other. For example, a promoter is operably linked or connected to a coding sequence if it controls the transcription of the
15 sequence. Amplification vectors do not require expression control domains, but rather need only the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. The need for control sequences in the expression vector will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional
20 operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding and sequences which control the termination of transcription and translation.

Preferred vectors preferably contain a promoter that is recognized by the host organism. The promoter sequences of the present invention may be prokaryotic, eukaryotic or viral. Examples of suitable prokaryotic sequences include the P_R and P_L promoters of
25 bacteriophage lambda (The bacteriophage Lambda, Hershey, A. D., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973), which is incorporated herein by reference in its entirety; Lambda II, Hendrix, R. W., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1980), which is incorporated herein by reference in its entirety); the *trp*, *recA*, heat shock, and *lacZ* promoters of *E. coli* and the SV40 early promoter (Benoist *et al. Nature*, 1981, 290,
30 304-310, which is incorporated herein by reference in its entirety). Additional promoters include, but are not limited to, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, Rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metallothionein.

Additional regulatory sequences can also be included in preferred vectors. Preferred examples of suitable regulatory sequences are represented by the Shine-Dalgarno of the replicase gene of the phage MS-2 and of the gene cII of bacteriophage lambda. The Shine-Dalgarno sequence may be directly followed by DNA encoding nGPCR-x and result in the
5 expression of the mature nGPCR-x protein.

Moreover, suitable expression vectors can include an appropriate marker that allows the screening of the transformed host cells. The transformation of the selected host is carried out using any one of the various techniques well known to the expert in the art and described in Sambrook *et al.*, *supra*.

10 An origin of replication can also be provided either by construction of the vector to include an exogenous origin or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient. Alternatively, rather than using vectors which contain viral origins of replication, one skilled in the art can transform mammalian cells by the method of co-transformation with
15 a selectable marker and nGPCR-x DNA. An example of a suitable marker is dihydrofolate reductase (DHFR) or thymidine kinase (*see*, U.S. Patent No. 4,399,216).

Nucleotide sequences encoding GPCR-x may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive
20 ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Sambrook *et al.*, *supra* and are well known in the art. Methods for construction of mammalian expression vectors are disclosed in, for example, Okayama *et al.*, *Mol. Cell. Biol.*, 1983, 3, 280, Cosman *et al.*, *Mol. Immunol.*, 1986, 23, 935, Cosman *et al.*, *Nature*,
25 1984, 312, 768, EP-A-0367566, and WO 91/18982, each of which is incorporated herein by reference in its entirety.

Host cells

According to another aspect of the invention, host cells are provided, including prokaryotic and eukaryotic cells, comprising a polynucleotide of the invention (or vector of
30 the invention) in a manner that permits expression of the encoded nGPCR-x polypeptide. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell that are well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion

with carriers such as liposomes, micelles, ghost cells, and protoplasts. Expression systems of the invention include bacterial, yeast, fungal, plant, insect, invertebrate, vertebrate, and mammalian cells systems.

5 The invention provides host cells that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the nGPCR-x polypeptide or fragment thereof encoded by the polynucleotide.

10 In still another related embodiment, the invention provides a method for producing a nGPCR-x polypeptide (or fragment thereof) comprising the steps of growing a host cell of the invention in a nutrient medium and isolating the polypeptide or variant thereof from the cell or the medium. Because nGPCR-x is a seven transmembrane receptor, it will be appreciated that, for some applications, such as certain activity assays, the preferable isolation may involve isolation of cell membranes containing the polypeptide embedded therein, whereas for other applications a more complete isolation may be preferable.

15 According to some aspects of the present invention, transformed host cells having an expression vector comprising any of the nucleic acid molecules described above are provided. Expression of the nucleotide sequence occurs when the expression vector is introduced into an appropriate host cell. Suitable host cells for expression of the polypeptides of the invention include, but are not limited to, prokaryotes, yeast, and eukaryotes. If a
20 prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Suitable prokaryotic cells include, but are not limited to, bacteria of the genera *Escherichia*, *Bacillus*, *Salmonella*, *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

25 If an eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence. Preferably, eukaryotic cells are cells of higher eukaryotes. Suitable eukaryotic cells include, but are not limited to, non-human mammalian tissue culture cells and human tissue culture cells. Preferred host cells include, but are not limited to, insect cells, HeLa cells, Chinese hamster ovary cells (CHO cells), African green monkey kidney cells (COS cells), human 293 cells, and murine
30 3T3 fibroblasts. Propagation of such cells in cell culture has become a routine procedure (see, Tissue Culture, Academic Press, Kruse and Patterson, eds. (1973), which is incorporated herein by reference in its entirety).

In addition, a yeast host may be employed as a host cell. Preferred yeast cells include, but are not limited to, the genera *Saccharomyces*, *Pichia*, and *Kluveromyces*. Preferred yeast

hosts are *S. cerevisiae* and *P. pastoris*. Preferred yeast vectors can contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replication sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Shuttle vectors for replication in both yeast and *E. coli* are also
5 included herein.

Alternatively, insect cells may be used as host cells. In a preferred embodiment, the polypeptides of the invention are expressed using a baculovirus expression system (*see* Luckow *et al.*, *Bio/Technology*, 1988, 6, 47, Baculovirus Expression Vectors: A Laboratory Manual, O'Rielly *et al.* (Eds.), W.H. Freeman and Company, New York, 1992, and U.S.
10 Patent No. 4,879,236, each of which is incorporated herein by reference in its entirety). In addition, the MAXBAC™ complete baculovirus expression system (Invitrogen) can, for example, be used for production in insect cells.

Host cells of the invention are a valuable source of immunogen for development of antibodies specifically immunoreactive with nGPCR-x. Host cells of the invention are also
15 useful in methods for the large-scale production of nGPCR-x polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells, or from the medium in which the cells are grown, by purification methods known in the art, *e.g.*, conventional chromatographic methods including immunoaffinity chromatography, receptor affinity chromatography, hydrophobic interaction chromatography,
20 lectin affinity chromatography, size exclusion filtration, cation or anion exchange chromatography, high pressure liquid chromatography (HPLC), reverse phase HPLC, and the like. Still other methods of purification include those methods wherein the desired protein is expressed and purified as a fusion protein having a specific tag, label, or chelating moiety that is recognized by a specific binding partner or agent. The purified protein can be cleaved
25 to yield the desired protein, or can be left as an intact fusion protein. Cleavage of the fusion component may produce a form of the desired protein having additional amino acid residues as a result of the cleavage process.

Knowledge of nGPCR-x DNA sequences allows for modification of cells to permit, or increase, expression of endogenous nGPCR-x. Cells can be modified (*e.g.*, by
30 homologous recombination) to provide increased expression by replacing, in whole or in part, the naturally occurring nGPCR-x promoter with all or part of a heterologous promoter so that the cells express nGPCR-x at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to endogenous nGPCR-x encoding sequences. (See, for example, PCT International Publication No. WO 94/12650, PCT International Publication

No. WO 92/20808, and PCT International Publication No. WO 91/09955.) It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.*, *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamoyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along
5 with the heterologous promoter DNA. If linked to the nGPCR-x coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the nGPCR-x coding sequences in the cells.

Knock-outs

The DNA sequence information provided by the present invention also makes
10 possible the development (*e.g.*, by homologous recombination or "knock-out" strategies; see Capecchi, *Science* 244:1288-1292 (1989), which is incorporated herein by reference) of animals that fail to express functional nGPCR-x or that express a variant of nGPCR-x. Such animals (especially small laboratory animals such as rats, rabbits, and mice) are useful as models for studying the *in vivo* activities of nGPCR-x and modulators of nGPCR-x.

Antisense

Also made available by the invention are anti-sense polynucleotides that recognize and hybridize to polynucleotides encoding nGPCR-x. Full-length and fragment anti-sense polynucleotides are provided. Fragment antisense molecules of the invention include (i)
20 those that specifically recognize and hybridize to nGPCR-x RNA (as determined by sequence comparison of DNA encoding nGPCR-x to DNA encoding other known molecules). Identification of sequences unique to nGPCR-x encoding polynucleotides can be deduced through use of any publicly available sequence database, and/or through use of commercially available sequence comparison programs. After identification of the desired sequences, isolation through restriction digestion or amplification using any of the various polymerase
25 chain reaction techniques well known in the art can be performed. Anti-sense polynucleotides are particularly relevant to regulating expression of nGPCR-x by those cells expressing nGPCR-x mRNA.

Antisense nucleic acids (preferably 10 to 30 base-pair oligonucleotides) capable of specifically binding to nGPCR-x expression control sequences or nGPCR-x RNA are
30 introduced into cells (*e.g.*, by a viral vector or colloidal dispersion system such as a liposome). The antisense nucleic acid binds to the nGPCR-x target nucleotide sequence in the cell and prevents transcription and/or translation of the target sequence. Phosphorothioate and methylphosphonate antisense oligonucleotides are specifically contemplated for therapeutic use by the invention. The antisense oligonucleotides may be further modified by

adding poly-L-lysine, transferrin polylysine, or cholesterol moieties at their 5' end.

Suppression of nGPCR-x expression at either the transcriptional or translational level is useful to generate cellular or animal models for diseases/conditions characterized by aberrant nGPCR-x expression.

5 Antisense oligonucleotides, or fragments of odd numbered nucleotide sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO:191, or sequences complementary or homologous thereto, derived from the nucleotide sequences of the present invention encoding nGPCR-x are useful as diagnostic tools for probing gene expression in various tissues. For example, tissue can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques to investigate native expression of this enzyme or pathological conditions relating thereto. Antisense oligonucleotides are preferably directed to regulatory regions of odd numbered nucleotide sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO:191, or mRNA corresponding thereto, including, but not limited to, the initiation codon, 10 TATA box, enhancer sequences, and the like.

Transcription factors

The nGPCR-x sequences taught in the present invention facilitate the design of novel transcription factors for modulating nGPCR-x expression in native cells and animals, and cells transformed or transfected with nGPCR-x polynucleotides. For example, the Cys₂-His₂ 20 zinc finger proteins, which bind DNA via their zinc finger domains, have been shown to be amenable to structural changes that lead to the recognition of different target sequences. These artificial zinc finger proteins recognize specific target sites with high affinity and low dissociation constants, and are able to act as gene switches to modulate gene expression. Knowledge of the particular nGPCR-x target sequence of the present invention facilitates the engineering of zinc finger proteins specific for the target sequence using known methods 25 such as a combination of structure-based modeling and screening of phage display libraries (Segal *et al.*, Proc. Natl. Acad. Sci. (USA) 96:2758-2763 (1999); Liu *et al.*, Proc. Natl. Acad. Sci. (USA) 94:5525-5530 (1997); Greisman *et al.*, Science 275:657-661 (1997); Choo *et al.*, J. Mol. Biol. 273:525-532 (1997)). Each zinc finger domain usually recognizes three or more 30 base pairs. Since a recognition sequence of 18 base pairs is generally sufficient in length to render it unique in any known genome, a zinc finger protein consisting of 6 tandem repeats of zinc fingers would be expected to ensure specificity for a particular sequence (Segal *et al.*) The artificial zinc finger repeats, designed based on nGPCR-x sequences, are fused to activation or repression domains to promote or suppress nGPCR-x expression (Liu *et al.*)

Alternatively, the zinc finger domains can be fused to the TATA box-binding factor (TBP) with varying lengths of linker region between the zinc finger peptide and the TBP to create either transcriptional activators or repressors (Kim *et al.*, Proc. Natl. Acad. Sci. (USA) 94:3616-3620 (1997). Such proteins and polynucleotides that encode them, have utility for
5 modulating nGPCR-x expression *in vivo* in both native cells, animals and humans; and/or cells transfected with nGPCR-x-encoding sequences. The novel transcription factor can be delivered to the target cells by transfecting constructs that express the transcription factor (gene therapy), or by introducing the protein. Engineered zinc finger proteins can also be designed to bind RNA sequences for use in therapeutics as alternatives to antisense or
10 catalytic RNA methods (McColl *et al.*, Proc. Natl. Acad. Sci. (USA) 96:9521-9526 (1997); Wu *et al.*, Proc. Natl. Acad. Sci. (USA) 92:344-348 (1995)). The present invention contemplates methods of designing such transcription factors based on the gene sequence of the invention, as well as customized zinc finger proteins, that are useful to modulate nGPCR-x expression in cells (native or transformed) whose genetic complement includes these
15 sequences.

Polypeptides

The invention also provides purified and isolated mammalian nGPCR-x polypeptides encoded by a polynucleotide of the invention. Presently preferred is a human nGPCR-x polypeptide comprising the amino acid sequence set out in even numbered sequences ranging
20 from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192, or fragments thereof comprising an epitope specific to the polypeptide. By "epitope specific to" is meant a portion of the nGPCR receptor that is recognizable by an antibody that is specific for the nGPCR, as defined in detail below.

Although the sequences provided are particular human sequences, the invention is
25 intended to include within its scope other human allelic variants; non-human mammalian forms of nGPCR-x, and other vertebrate forms of nGPCR-x.

It will be appreciated that extracellular epitopes are particularly useful for generating and screening for antibodies and other binding compounds that bind to receptors such as nGPCR-x. Thus, in another preferred embodiment, the invention provides a purified and
30 isolated polypeptide comprising at least one extracellular domain (*e.g.*, the N-terminal extracellular domain or one of the three extracellular loops) of nGPCR-x. Purified and isolated polypeptides comprising the N-terminal extracellular domain of nGPCR-x are highly preferred. Also preferred is a purified and isolated polypeptide comprising a nGPCR-x fragment selected from the group consisting of the N-terminal extracellular domain of

nGPCR-x, transmembrane domains of nGPCR-x, an extracellular loop connecting transmembrane domains of nGPCR-x, an intracellular loop connecting transmembrane domains of nGPCR-x, the C-terminal cytoplasmic region of nGPCR-x, and fusions thereof. Such fragments may be continuous portions of the native receptor. However, it will also be appreciated that knowledge of the nGPCR-x gene and protein sequences as provided herein permits recombining of various domains that are not contiguous in the native protein. Using a FORTRAN computer program called "tmtrest.all" [Parodi *et al.*, Comput. Appl. Biosci. 5:527-535 (1994)], nGPCR-x was shown to contain transmembrane-spanning domains.

The invention also embraces polypeptides that have at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55% or at least 50% identity and/or homology to the preferred polypeptide of the invention. Percent amino acid sequence "identity" with respect to the preferred polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the nGPCR-x sequence after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence "homology" with respect to the preferred polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the nGPCR-x sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and also considering any conservative substitutions as part of the sequence identity.

In one aspect, percent homology is calculated as the percentage of amino acid residues in the smaller of two sequences which align with identical amino acid residue in the sequence being compared, when four gaps in a length of 100 amino acids may be introduced to maximize alignment [Dayhoff, in Atlas of Protein Sequence and Structure, Vol. 5, p. 124, National Biochemical Research Foundation, Washington, D.C. (1972), incorporated herein by reference].

Polypeptides of the invention may be isolated from natural cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation, lipidation, and phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of nGPCR-x polypeptides are embraced by the invention.

The invention also embraces variant (or analog) nGPCR-x polypeptides. In one example, insertion variants are provided wherein one or more amino acid residues supplement a nGPCR-x amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the nGPCR-x amino acid sequence. Insertional variants with additional residues at either or both termini can include, for example, fusion proteins and proteins including amino acid tags or labels.

Insertion variants include nGPCR-x polypeptides wherein one or more amino acid residues are added to a nGPCR-x acid sequence or to a biologically active fragment thereof.

Variant products of the invention also include mature nGPCR-x products, *i.e.*, nGPCR-x products wherein leader or signal sequences are removed, with additional amino terminal residues. The additional amino terminal residues may be derived from another protein, or may include one or more residues that are not identifiable as being derived from specific proteins. nGPCR-x products with an additional methionine residue at position -1 (Met⁻¹-nGPCR-x) are contemplated, as are variants with additional methionine and lysine residues at positions -2 and -1 (Met⁻²-Lys⁻¹-nGPCR-x). Variants of nGPCR-x with additional Met, Met-Lys, Lys residues (or, one or more basic residues in general) are particularly useful for enhanced recombinant protein production in bacterial host cells.

The invention also embraces nGPCR-x variants having additional amino acid residues that result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of a glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants that result from expression in other vector systems are also contemplated.

Insertional variants also include fusion proteins wherein the amino terminus and/or the carboxy terminus of nGPCR-x is/are fused to another polypeptide.

In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a nGPCR-x polypeptide are removed. Deletions can be effected at one or both termini of the nGPCR-x polypeptide, or with removal of one or more non-terminal amino acid residues of nGPCR-x. Deletion variants, therefore, include all fragments of a nGPCR-x polypeptide.

The invention also embraces polypeptide fragments of the even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192, wherein the fragments maintain biological (*e.g.*, ligand binding and/or intracellular signaling) immunological properties of a nGPCR-x polypeptide.

In one preferred embodiment of the invention, an isolated nucleic acid molecule comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to even numbered sequences selected from the group consisting of: SEQ ID NO:2 to SEQ ID NO:94, SEQ ID NO: 186 and SEQ ID NO:192, and fragments thereof, wherein the nucleic acid molecule encoding at least a portion of nGPCR-x. In a more preferred embodiment, the isolated nucleic acid molecule comprises a sequence that encodes a polypeptide comprising even numbered sequences selected from the group consisting of SEQ ID NO:2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192, and fragments thereof.

As used in the present invention, polypeptide fragments comprise at least 5, 10, 15, 20, 25, 30, 35, or 40 consecutive amino acids of the even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192. Preferred polypeptide fragments display antigenic properties unique to, or specific for, human nGPCR-x and its allelic and species homologs. Fragments of the invention having the desired biological and immunological properties can be prepared by any of the methods well known and routinely practiced in the art.

In still another aspect, the invention provides substitution variants of nGPCR-x polypeptides. Substitution variants include those polypeptides wherein one or more amino acid residues of a nGPCR-x polypeptide are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Conservative substitutions for this purpose may be defined as set out in Tables 2, 3, or 4 below.

Variant polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention.

Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table 2 (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96), immediately below.

Table 2

Conservative Substitutions I

| <u>SIDE CHAIN CHARACTERISTIC</u> | <u>AMINO ACID</u> |
|--------------------------------------|-------------------|
| Aliphatic | |
| Non-polar | G A P I L V |
| Polar - uncharged | C S T M N Q |
| Polar - charged | D E K R |
| Aromatic | H F W Y |
| Other | N Q D E |

Alternatively, conservative amino acids can be grouped as described in Lehninger,
 5 [Biochemistry, Second Edition; Worth Publishers, Inc. NY, NY (1975), pp.71-77] as set out
 in Table 3, below.

Table 3
Conservative Substitutions II

10

| <u>SIDE CHAIN CHARACTERISTIC</u> | <u>AMINO ACID</u> |
|--------------------------------------|-------------------|
| Non-polar (hydrophobic) | |
| A. Aliphatic: | A L I V P |
| B. Aromatic: | F W |
| C. Sulfur-containing: | M |
| D. Borderline: | G |
| Uncharged-polar | |
| A. Hydroxyl: | S T Y |
| B. Amides: | N Q |
| C. Sulfhydryl: | C |
| D. Borderline: | G |
| Positively Charged (Basic): | K R H |
| Negatively Charged (Acidic): | D E |

As still another alternative, exemplary conservative substitutions are set out in Table
 4, below.

15

Table 4
Conservative Substitutions III

| <u>Original Residue</u> | <u>Exemplary Substitution</u> |
|-------------------------|-------------------------------|
| Ala (A) | Val, Leu, Ile |
| Arg (R) | Lys, Gln, Asn |
| Asn (N) | Gln, His, Lys, Arg |
| Asp (D) | Glu |
| Cys (C) | Ser |
| Gln (Q) | Asn |
| Glu (E) | Asp |
| His (H) | Asn, Gln, Lys, Arg |

| | |
|---------|--------------------------|
| Ile (I) | Leu, Val, Met, Ala, Phe, |
| Leu (L) | Ile, Val, Met, Ala, Phe |
| Lys (K) | Arg, Gln, Asn |
| Met (M) | Leu, Phe, Ile |
| Phe (F) | Leu, Val, Ile, Ala |
| Pro (P) | Gly |
| Ser (S) | Thr |
| Thr (T) | Ser |
| Trp (W) | Tyr |
| Tyr (Y) | Trp, Phe, Thr, Ser |
| Val (V) | Ile, Leu, Met, Phe, Ala |

It should be understood that the definition of polypeptides of the invention is intended to include polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. By way of example, the modifications may be covalent in nature, and include for example, chemical bonding with polymers, lipids, other organic, and inorganic moieties. Such derivatives may be prepared to increase circulating half-life of a polypeptide, or may be designed to improve the targeting capacity of the polypeptide for desired cells, tissues, or organs. Similarly, the invention further embraces nGPCR-x polypeptides that have been covalently modified to include one or more water-soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. Variants that display ligand binding properties of native nGPCR-x and are expressed at higher levels, as well as variants that provide for constitutively active receptors, are particularly useful in assays of the invention; the variants are also useful in providing cellular, tissue and animal models of diseases/conditions characterized by aberrant nGPCR-x activity.

In a related embodiment, the present invention provides compositions comprising purified polypeptides of the invention. Preferred compositions comprise, in addition to the polypeptide of the invention, a pharmaceutically acceptable (*i.e.*, sterile and non-toxic) liquid, semisolid, or solid diluent that serves as a pharmaceutical vehicle, excipient, or medium. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, water, saline solutions, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, glycerol, calcium phosphate, mineral oil, and cocoa butter.

Variants that display ligand binding properties of native nGPCR-x and are expressed at higher levels, as well as variants that provide for constitutively active receptors, are particularly useful in assays of the invention; the variants are also useful in assays of the

invention and in providing cellular, tissue and animal models of diseases/conditions characterized by aberrant nGPCR-x activity.

The G protein-coupled receptor functions through a specific heterotrimeric guanine-nucleotide-binding regulatory protein (G-protein) coupled to the intracellular portion of the G protein-coupled receptor molecule. Accordingly, the G protein-coupled receptor has a specific affinity to G protein. G proteins specifically bind to guanine nucleotides. Isolation of G proteins provides a means to isolate guanine nucleotides. G Proteins may be isolated using commercially available anti-G protein antibodies or isolated G protein-coupled receptors. Similarly, G proteins may be detected in a sample isolated using commercially available detectable anti-G protein antibodies or isolated G protein-coupled receptors.

According to the present invention, the isolated n-GPCR-x proteins of the present invention are useful to isolate and purify G proteins from samples such as cell lysates. Example 15 below sets forth an example of isolation of G proteins using isolated nGPCR-x proteins. Such methodology may be used in place of the use of commercially available anti-G protein antibodies which are used to isolate G proteins. Moreover, G proteins may be detected using nGPCR-x proteins in place of commercially available detectable anti-G protein antibodies. Since nGPCR-x proteins specifically bind to G proteins, they can be employed in any specific use where G protein specific affinity is required, such as those uses where commercially available anti-G protein antibodies are employed.

Antibodies

Also comprehended by the present invention are antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) specific for nGPCR-x or fragments thereof. Preferred antibodies of the invention are human antibodies that are produced and identified according to methods described in WO93/11236, published June 20, 1993, which is incorporated herein by reference in its entirety. Antibody fragments, including Fab, Fab', F(ab')₂, and F_v, are also provided by the invention. The term "specific for," when used to describe antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and bind nGPCR-x polypeptides exclusively (i.e., are able to distinguish nGPCR-x polypeptides from other known GPCR polypeptides by virtue of measurable differences in binding affinity, despite the possible existence of localized sequence identity, homology, or similarity between nGPCR-x and such polypeptides). It will

be understood that specific antibodies may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and, in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow *et al.* (Eds.), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the nGPCR-x polypeptides of the invention are also contemplated, provided that the antibodies are specific for nGPCR-x polypeptides.

10 Antibodies of the invention can be produced using any method well known and routinely practiced in the art.

The invention provides an antibody that is specific for the nGPCR-x of the invention. Antibody specificity is described in greater detail below. However, it should be emphasized that antibodies that can be generated from polypeptides that have previously been described in the literature and that are capable of fortuitously cross-reacting with nGPCR-x (e.g., due to the fortuitous existence of a similar epitope in both polypeptides) are considered "cross-reactive" antibodies. Such cross-reactive antibodies are not antibodies that are "specific" for nGPCR-x. The determination of whether an antibody is specific for nGPCR-x or is cross-reactive with another known receptor is made using any of several assays, such as Western blotting assays, that are well known in the art. For identifying cells that express nGPCR-x and also for modulating nGPCR-x-ligand binding activity, antibodies that specifically bind to an extracellular epitope of the nGPCR-x are preferred.

15 20

In one preferred variation, the invention provides monoclonal antibodies. Hybridomas that produce such antibodies also are intended as aspects of the invention. In yet another variation, the invention provides a humanized antibody. Humanized antibodies are useful for *in vivo* therapeutic indications.

25

In another variation, the invention provides a cell-free composition comprising polyclonal antibodies, wherein at least one of the antibodies is an antibody of the invention specific for nGPCR-x. Antisera isolated from an animal is an exemplary composition, as is a composition comprising an antibody fraction of an antisera that has been resuspended in water or in another diluent, excipient, or carrier.

30

In still another related embodiment, the invention provides an anti-idiotypic antibody specific for an antibody that is specific for nGPCR-x.

It is well known that antibodies contain relatively small antigen binding domains that can be isolated chemically or by recombinant techniques. Such domains are useful nGPCR-x binding molecules themselves, and also may be reintroduced into human antibodies, or fused to toxins or other polypeptides. Thus, in still another embodiment, the invention provides a polypeptide comprising a fragment of a nGPCR-x-specific antibody, wherein the fragment and the polypeptide bind to the nGPCR-x. By way of non-limiting example, the invention provides polypeptides that are single chain antibodies and CDR-grafted antibodies.

Non-human antibodies may be humanized by any of the methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

Antibodies of the invention are useful for, *e.g.*, therapeutic purposes (by modulating activity of nGPCR-x), diagnostic purposes to detect or quantitate nGPCR-x, and purification of nGPCR-x. Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific.

Compositions

Mutations in the nGPCR-x gene that result in loss of normal function of the nGPCR-x gene product underlie nGPCR-x-related human disease states. The invention comprehends gene therapy to restore nGPCR-x activity to treat those disease states. Delivery of a functional nGPCR-x gene to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (*e.g.*, adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (*e.g.*, liposomes or chemical treatments). See, for example, Anderson, *Nature*, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, *Science*, 244: 1275-1281 (1989); Verma, *Scientific American*: 68-84 (1990); and Miller, *Nature*, 357: 455-460 (1992). Alternatively, it is contemplated that in other human disease states, preventing the expression of, or inhibiting the activity of, nGPCR-x will be useful in treating disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of nGPCR-x.

Another aspect of the present invention is directed to compositions, including pharmaceutical compositions, comprising any of the nucleic acid molecules or recombinant expression vectors described above and an acceptable carrier or diluent. Preferably, the carrier or diluent is pharmaceutically acceptable. Suitable carriers are described in the most

recent edition of *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference in its entirety. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The formulations are sterilized by commonly used techniques.

Also within the scope of the invention are compositions comprising polypeptides, polynucleotides, or antibodies of the invention that have been formulated with, *e.g.*, a pharmaceutically acceptable carrier.

The invention also provides methods of using antibodies of the invention. For example, the invention provides a method for modulating ligand binding of a nGPCR-x comprising the step of contacting the nGPCR-x with an antibody specific for the nGPCR-x, under conditions wherein the antibody binds the receptor.

GPCRs that may be expressed in the brain, such as nGPCR-x, provide an indication that aberrant nGPCR-x signaling activity may correlate with one or more neurological or psychological disorders. The invention also provides a method for treating a neurological or psychiatric disorder comprising the step of administering to a mammal in need of such treatment an amount of an antibody-like polypeptide of the invention that is sufficient to modulate ligand binding to a nGPCR-x in neurons of the mammal. nGPCR-x may also be expressed in other tissues, including but not limited to, peripheral blood lymphocytes, pancreas, ovary, uterus, testis, salivary gland, thyroid gland, kidney, adrenal gland, liver, bone marrow, prostate, fetal liver, colon, muscle, and fetal brain, and may be found in many other tissues. Within the brain, nGPCR-x mRNA transcripts may be found in many tissues, including, but not limited to, frontal lobe, hypothalamus, pons, cerebellum, caudate nucleus, and medulla. Tissues and brain regions where specific nGPCRs of the present invention are expressed are identified in the Examples below.

Kits

The present invention is also directed to kits, including pharmaceutical kits. The kits can comprise any of the nucleic acid molecules described above, any of the polypeptides described above, or any antibody which binds to a polypeptide of the invention as described above, as well as a negative control. The kit preferably comprises additional components, such as, for example, instructions, solid support, reagents helpful for quantification, and the like.

In another aspect, the invention features methods for detection of a polypeptide in a sample as a diagnostic tool for diseases or disorders, wherein the method comprises the steps of: (a) contacting the sample with a nucleic acid probe which hybridizes under hybridization assay conditions to a nucleic acid target region of a polypeptide having the sequence of even
 5 numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192, said probe comprising the nucleic acid sequence encoding the polypeptide, fragments thereof, and the complements of the sequences and fragments; and (b) detecting the presence or amount of the probe:target region hybrid as an indication of the disease.

In preferred embodiments of the invention, the disease is selected from the group
 10 consisting of thyroid disorders (*e.g.* thyrotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Crohn's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder,
 15 post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, *etc.*); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases
 20 and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); and sexual dysfunction,
 25 among others.

As described above and in Example 4 below, the genes encoding nGPCR-1 (nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 73, amino acid sequence SEQ ID NO: 2, SEQ ID NO:74), nGPCR-9 (nucleic acid sequence SEQ ID NO:9, SEQ ID NO:77, amino acid sequence SEQ ID NO:10, SEQ ID NO:78), nGPCR-11 (nucleic acid sequence SEQ ID
 30 NO:11, SEQ ID NO:79, amino acid sequence SEQ ID NO:12, SEQ ID NO:80), nGPCR-16 (nucleic acid sequence SEQ ID NO: 21, SEQ ID NO:81, amino acid sequence SEQ ID NO: 22, SEQ ID NO:82), nGPCR-40 (nucleic acid sequence SEQ ID NO:53, SEQ ID NO:83, amino acid sequence SEQ ID NO:54, SEQ ID NO:84), nGPCR-54 (nucleic acid sequence SEQ ID NO:59, SEQ ID NO:85, amino acid sequence SEQ ID NO:60, SEQ ID NO: 86),

nGPCR-56 (nucleic acid sequence SEQ ID NO:63, SEQ ID NO:87, SEQ ID NO:89, amino acid sequence SEQ ID NO:64, SEQ ID NO: 88, SEQ ID NO:90), nGPCR-58 (nucleic acid sequence SEQ ID NO:67, SEQ ID NO:91, SEQ ID NO:93, amino acid sequence SEQ ID NO:68, SEQ ID NO: 92, SEQ ID NO:94) and nGPCR-3 (nucleic acid sequence SEQ ID NO:3, SEQ ID NO:185, amino acid sequence SEQ ID NO:4, SEQ ID NO: 186) have been detected in brain tissue indicating that these n-GPCR-x proteins are neuroreceptors. Kits may be designed to detect either expression of polynucleotides encoding these proteins or the proteins themselves in order to identify tissue as being neurological. For example, oligonucleotide hybridization kits can be provided which include a container having an oligonucleotide probe specific for the n-GPCR-x-specific DNA and optionally, containers with positive and negative controls and/or instructions. Similarly, PCR kits can be provided which include a container having primers specific for the n-GPCR-x-specific sequences, DNA and optionally, containers with size markers, positive and negative controls and/or instructions.

Hybridization conditions should be such that hybridization occurs only with the genes in the presence of other nucleic acid molecules. Under stringent hybridization conditions only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 1 or 2 mismatches out of 20 contiguous nucleotides. Such conditions are defined supra.

The diseases for which detection of genes in a sample could be diagnostic include diseases in which nucleic acid (DNA and/or RNA) is amplified in comparison to normal cells. By "amplification" is meant increased numbers of DNA or RNA in a cell compared with normal cells.

The diseases that could be diagnosed by detection of nucleic acid in a sample preferably include central nervous system and metabolic diseases. The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The samples used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample that is compatible with the method utilized.

Alternatively, immunoassay kits can be provided which have containers containing antibodies specific for the n-GPCR-x-protein and optionally, containers with positive and negative controls and/or instructions.

Kits may also be provided useful in the identification of GPCR binding partners such as natural ligands or modulators (agonists or antagonists). Substances useful for treatment of disorders or diseases preferably show positive results in one or more *in vitro* assays for an activity corresponding to treatment of the disease or disorder in question. Substances that
5 modulate the activity of the polypeptides preferably include, but are not limited to, antisense oligonucleotides, agonists and antagonists, and inhibitors of protein kinases.

Methods of inducing immune response

Another aspect of the present invention is directed to methods of inducing an immune response in a mammal against a polypeptide of the invention by administering to the mammal
10 an amount of the polypeptide sufficient to induce an immune response. The amount will be dependent on the animal species, size of the animal, and the like but can be determined by those skilled in the art.

Methods of identifying ligands

The invention also provides assays to identify compounds that bind nGPCR-x. One
15 such assay comprises the steps of: (a) contacting a composition comprising a nGPCR-x with a compound suspected of binding nGPCR-x; and (b) measuring binding between the compound and nGPCR-x. In one variation, the composition comprises a cell expressing nGPCR-x on its surface. In another variation, isolated nGPCR-x or cell membranes comprising nGPCR-x are employed. The binding may be measured directly, *e.g.*, by using a
20 labeled compound, or may be measured indirectly by several techniques, including measuring intracellular signaling of nGPCR-x induced by the compound (or measuring changes in the level of nGPCR-x signaling).

Specific binding molecules, including natural ligands and synthetic compounds, can be identified or developed using isolated or recombinant nGPCR-x products, nGPCR-x
25 variants, or preferably, cells expressing such products. Binding partners are useful for purifying nGPCR-x products and detection or quantification of nGPCR-x products in fluid and tissue samples using known immunological procedures. Binding molecules are also manifestly useful in modulating (*i.e.*, blocking, inhibiting or stimulating) biological activities of nGPCR-x, especially those activities involved in signal transduction.

30 The DNA and amino acid sequence information provided by the present invention also makes possible identification of binding partner compounds with which a nGPCR-x polypeptide or polynucleotide will interact. Methods to identify binding partner compounds include solution assays, *in vitro* assays wherein nGPCR-x polypeptides are immobilized, and cell-based assays. Identification of binding partner compounds of nGPCR-x polypeptides

provides candidates for therapeutic or prophylactic intervention in pathologies associated with nGPCR-x normal and aberrant biological activity.

The invention includes several assay systems for identifying nGPCR-x binding partners. In solution assays, methods of the invention comprise the steps of (a) contacting a
5 nGPCR-x polypeptide with one or more candidate binding partner compounds and (b) identifying the compounds that bind to the nGPCR-x polypeptide. Identification of the compounds that bind the nGPCR-x polypeptide can be achieved by isolating the nGPCR-x polypeptide/binding partner complex, and separating the binding partner compound from the nGPCR-x polypeptide. An additional step of characterizing the physical, biological, and/or
10 biochemical properties of the binding partner compound is also comprehended in another embodiment of the invention. In one aspect, the nGPCR-x polypeptide/binding partner complex is isolated using an antibody immunospecific for either the nGPCR-x polypeptide or the candidate binding partner compound.

In still other embodiments, either the nGPCR-x polypeptide or the candidate binding
15 partner compound comprises a label or tag that facilitates its isolation, and methods of the invention to identify binding partner compounds include a step of isolating the nGPCR-x polypeptide/binding partner complex through interaction with the label or tag. An exemplary tag of this type is a poly-histidine sequence, generally around six histidine residues, that permits isolation of a compound so labeled using nickel chelation. Other labels and tags,
20 such as the FLAG® tag (Eastman Kodak, Rochester, NY), well known and routinely used in the art, are embraced by the invention.

In one variation of an *in vitro* assay, the invention provides a method comprising the steps of (a) contacting an immobilized nGPCR-x polypeptide with a candidate binding partner compound and (b) detecting binding of the candidate compound to the nGPCR-x
25 polypeptide. In an alternative embodiment, the candidate binding partner compound is immobilized and binding of nGPCR-x is detected. Immobilization is accomplished using any of the methods well known in the art, including covalent bonding to a support, a bead, or a chromatographic resin, as well as non-covalent, high affinity interactions such as antibody binding, or use of streptavidin/biotin binding wherein the immobilized compound includes a
30 biotin moiety. Detection of binding can be accomplished (i) using a radioactive label on the compound that is not immobilized, (ii) using of a fluorescent label on the non-immobilized compound, (iii) using an antibody immunospecific for the non-immobilized compound, (iv) using a label on the non-immobilized compound that excites a fluorescent support to which

the immobilized compound is attached, as well as other techniques well known and routinely practiced in the art.

The invention also provides cell-based assays to identify binding partner compounds of a nGPCR-x polypeptide. In one embodiment, the invention provides a method comprising
5 the steps of contacting a nGPCR-x polypeptide expressed on the surface of a cell with a candidate binding partner compound and detecting binding of the candidate binding partner compound to the nGPCR-x polypeptide. In a preferred embodiment, the detection comprises detecting a calcium flux or other physiological event in the cell caused by the binding of the molecule.

10 Another aspect of the present invention is directed to methods of identifying compounds that bind to either nGPCR-x or nucleic acid molecules encoding nGPCR-x, comprising contacting nGPCR-x, or a nucleic acid molecule encoding the same, with a compound, and determining whether the compound binds nGPCR-x or a nucleic acid molecule encoding the same. Binding can be determined by binding assays which are well
15 known to the skilled artisan, including, but not limited to, gel-shift assays, Western blots, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, ELISA, and the like, which are described in, for example, *Current Protocols in Molecular Biology*, 1999, John Wiley & Sons, NY, which is incorporated herein
20 by reference in its entirety. The compounds to be screened include (which may include compounds which are suspected to bind nGPCR-x, or a nucleic acid molecule encoding the same), but are not limited to, extracellular, intracellular, biologic or chemical origin. The methods of the invention also embrace ligands, especially neuropeptides, that are attached to a label, such as a radiolabel (*e.g.*, ^{125}I , ^{35}S , ^{32}P , ^{33}P , ^3H), a fluorescence label, a
25 chemiluminescent label, an enzymic label and an immunogenic label. Modulators falling within the scope of the invention include, but are not limited to, non-peptide molecules such as non-peptide mimetics, non-peptide allosteric effectors, and peptides. The nGPCR-x polypeptide or polynucleotide employed in such a test may either be free in solution, attached to a solid support, borne on a cell surface or located intracellularly or associated with a
30 portion of a cell. One skilled in the art can, for example, measure the formation of complexes between nGPCR-x and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between nGPCR-x and its substrate caused by the compound being tested.

In another embodiment of the invention, high throughput screening for compounds having suitable binding affinity to nGPCR-x is employed. Briefly, large numbers of different small peptide test compounds are synthesized on a solid substrate. The peptide test compounds are contacted with nGPCR-x and washed. Bound nGPCR-x is then detected by methods well known in the art. Purified polypeptides of the invention can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the protein and immobilize it on the solid support.

Generally, an expressed nGPCR-x can be used for HTS binding assays in conjunction with its defined ligand, in this case the corresponding neuropeptide that activates it. The identified peptide is labeled with a suitable radioisotope, including, but not limited to, ^{125}I , ^3H , ^{35}S or ^{32}P , by methods that are well known to those skilled in the art. Alternatively, the peptides may be labeled by well-known methods with a suitable fluorescent derivative (Baindur *et al.*, *Drug Dev. Res.*, 1994, 33, 373-398; Rogers, *Drug Discovery Today*, 1997, 2, 156-160). Radioactive ligand specifically bound to the receptor in membrane preparations made from the cell line expressing the recombinant protein can be detected in HTS assays in one of several standard ways, including filtration of the receptor-ligand complex to separate bound ligand from unbound ligand (Williams, *Med. Res. Rev.*, 1991, 11, 147-184; Sweetnam *et al.*, *J. Natural Products*, 1993, 56, 441-455). Alternative methods include a scintillation proximity assay (SPA) or a FlashPlate format in which such separation is unnecessary (Nakayama, *Cur. Opinion Drug Disc. Dev.*, 1998, 1, 85-91 Bossé *et al.*, *J. Biomolecular Screening*, 1998, 3, 285-292.). Binding of fluorescent ligands can be detected in various ways, including fluorescence energy transfer (FRET), direct spectrophotofluorometric analysis of bound ligand, or fluorescence polarization (Rogers, *Drug Discovery Today*, 1997, 2, 156-160; Hill, *Cur. Opinion Drug Disc. Dev.*, 1998, 1, 92-97).

Other assays may be used to identify specific ligands of a nGPCR-x receptor, including assays that identify ligands of the target protein through measuring direct binding of test ligands to the target protein, as well as assays that identify ligands of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two-hybrid system described in Fields *et al.*, *Nature*, 340:245-246 (1989), and Fields *et al.*, *Trends in Genetics*, 10:286-292 (1994), both of which are incorporated herein by reference. The two-hybrid system is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that

bind to a known protein of interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA binding proteins, to identify peptides that bind to a protein, and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA binding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein and (2) an activation domain fused to a second protein. The DNA-binding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid proteins are present, the noncovalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene. For example, when the first protein is a GPCR gene product, or fragment thereof, that is known to interact with another protein or nucleic acid, this assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system. The presence of an inhibitory agent results in lack of a reporter signal.

The function of nGPCR-x gene products is unclear and no ligands have yet been found which bind the gene product. The yeast two-hybrid assay can also be used to identify proteins that bind to the gene product. In an assay to identify proteins that bind to a nGPCR-x receptor, or fragment thereof, a fusion polynucleotide encoding both a nGPCR-x receptor (or fragment) and a UAS binding domain (*i.e.*, a first protein) may be used. In addition, a large number of hybrid genes each encoding a different second protein fused to an activation domain are produced and screened in the assay. Typically, the second protein is encoded by one or more members of a total cDNA or genomic DNA fusion library, with each second protein-coding region being fused to the activation domain. This system is applicable to a wide variety of proteins, and it is not even necessary to know the identity or function of the second binding protein. The system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein.

Other assays may be used to search for agents that bind to the target protein. One such screening method to identify direct binding of test ligands to a target protein is described

in U.S. Patent No. 5,585,277, incorporated herein by reference. This method relies on the principle that proteins generally exist as a mixture of folded and unfolded states, and continually alternate between the two states. When a test ligand binds to the folded form of a target protein (i.e., when the test ligand is a ligand of the target protein), the target protein molecule bound by the ligand remains in its folded state. Thus, the folded target protein is present to a greater extent in the presence of a test ligand which binds the target protein, than in the absence of a ligand. Binding of the ligand to the target protein can be determined by any method that distinguishes between the folded and unfolded states of the target protein. The function of the target protein need not be known in order for this assay to be performed. Virtually any agent can be assessed by this method as a test ligand, including, but not limited to, metals, polypeptides, proteins, lipids, polysaccharides, polynucleotides and small organic molecules.

Another method for identifying ligands of a target protein is described in Wieboldt *et al.*, *Anal. Chem.*, 69:1683-1691 (1997), incorporated herein by reference. This technique screens combinatorial libraries of 20-30 agents at a time in solution phase for binding to the target protein. Agents that bind to the target protein are separated from other library components by simple membrane washing. The specifically selected molecules that are retained on the filter are subsequently liberated from the target protein and analyzed by HPLC and pneumatically assisted electrospray (ion spray) ionization mass spectroscopy. This procedure selects library components with the greatest affinity for the target protein, and is particularly useful for small molecule libraries.

Other embodiments of the invention comprise using competitive screening assays in which neutralizing antibodies capable of binding a polypeptide of the invention specifically compete with a test compound for binding to the polypeptide. In this manner, the antibodies can be used to detect the presence of any peptide that shares one or more antigenic determinants with nGPCR-x. Radiolabeled competitive binding studies are described in A.H. Lin *et al. Antimicrobial Agents and Chemotherapy*, 1997, vol. 41, no. 10. pp. 2127-2131, the disclosure of which is incorporated herein by reference in its entirety.

As described above and in Example 4 below, the genes encoding nGPCR-1 (nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 73, amino acid sequence SEQ ID NO: 2, SEQ ID NO: 74), nGPCR-9 (nucleic acid sequence SEQ ID NO: 9, SEQ ID NO: 77, amino acid sequence SEQ ID NO: 10, SEQ ID NO: 78), nGPCR-11 (nucleic acid sequence SEQ ID NO: 11, SEQ ID NO: 79, amino acid sequence SEQ ID NO: 12, SEQ ID NO: 80), nGPCR-16 (nucleic acid sequence SEQ ID NO: 21, SEQ ID NO: 81, amino acid sequence SEQ ID NO:

22, SEQ ID NO:82), nGPCR-40 (nucleic acid sequence SEQ ID NO:53, SEQ ID NO:83, amino acid sequence SEQ ID NO:54, SEQ ID NO:84), nGPCR-54 (nucleic acid sequence SEQ ID NO:59, SEQ ID NO:85, amino acid sequence SEQ ID NO:60, SEQ ID NO: 86), nGPCR-56 (nucleic acid sequence SEQ ID NO:63, SEQ ID NO:87, SEQ ID NO:89, amino acid sequence SEQ ID NO:64, SEQ ID NO: 88, SEQ ID NO:90), nGPCR-58 (nucleic acid sequence SEQ ID NO:67, SEQ ID NO:91, SEQ ID NO:93, amino acid sequence SEQ ID NO:68, SEQ ID NO: 92, SEQ ID NO:94), and nGPCR-3 (nucleic acid sequence SEQ ID NO:3, SEQ ID NO:185, amino acid sequence SEQ ID NO:4, SEQ ID NO: 186) have been detected in brain tissue indicating that these n-GPCR-x proteins are neuroreceptors.

10 Accordingly, natural binding partners of these molecules include neurotransmitters.

Identification of modulating agents

The invention also provides methods for identifying a modulator of binding between a nGPCR-x and a nGPCR-x binding partner, comprising the steps of: (a) contacting a nGPCR-x binding partner and a composition comprising a nGPCR-x in the presence and in the absence of a putative modulator compound; (b) detecting binding between the binding partner and the nGPCR-x; and (c) identifying a putative modulator compound or a modulator compound in view of decreased or increased binding between the binding partner and the nGPCR-x in the presence of the putative modulator, as compared to binding in the absence of the putative modulator.

20 nGPCR-x binding partners that stimulate nGPCR-x activity are useful as agonists in disease states or conditions characterized by insufficient nGPCR-x signaling (*e.g.*, as a result of insufficient activity of a nGPCR-x ligand). nGPCR-x binding partners that block ligand-mediated nGPCR-x signaling are useful as nGPCR-x antagonists to treat disease states or conditions characterized by excessive nGPCR-x signaling. In addition nGPCR-x modulators in general, as well as nGPCR-x polynucleotides and polypeptides, are useful in diagnostic assays for such diseases or conditions.

In another aspect, the invention provides methods for treating a disease or abnormal condition by administering to a patient in need of such treatment a substance that modulates the activity or expression of a polypeptide having the sequence of even numbered sequences ranging from SEQ ID NO: 2 to SEQ ID NO: 94, SEQ ID NO: 186 and SEQ ID NO:192.

30 Agents that modulate (*i.e.*, increase, decrease, or block) nGPCR-x activity or expression may be identified by incubating a putative modulator with a cell containing a nGPCR-x polypeptide or polynucleotide and determining the effect of the putative modulator on nGPCR-x activity or expression. The selectivity of a compound that modulates the

activity of nGPCR-x can be evaluated by comparing its effects on nGPCR-x to its effect on other GPCR compounds. Selective modulators may include, for example, antibodies and other proteins, peptides, or organic molecules that specifically bind to a nGPCR-x polypeptide or a nGPCR-x-encoding nucleic acid. Modulators of nGPCR-x activity will be therapeutically useful in treatment of diseases and physiological conditions in which normal or aberrant nGPCR-x activity is involved. nGPCR-x polynucleotides, polypeptides, and modulators may be used in the treatment of such diseases and conditions as infections, such as viral infections caused by HIV-1 or HIV-2; pain; cancers; Parkinson's disease; hypotension; hypertension; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Tourette's Syndrome, among others. nGPCR-x polynucleotides and polypeptides, as well as nGPCR-x modulators, may also be used in diagnostic assays for such diseases or conditions.

Methods of the invention to identify modulators include variations on any of the methods described above to identify binding partner compounds, the variations including techniques wherein a binding partner compound has been identified and the binding assay is carried out in the presence and absence of a candidate modulator. A modulator is identified in those instances where binding between the nGPCR-x polypeptide and the binding partner compound changes in the presence of the candidate modulator compared to binding in the absence of the candidate modulator compound. A modulator that increases binding between the nGPCR-x polypeptide and the binding partner compound is described as an enhancer or activator, and a modulator that decreases binding between the nGPCR-x polypeptide and the binding partner compound is described as an inhibitor.

The invention also comprehends high-throughput screening (HTS) assays to identify compounds that interact with or inhibit biological activity (*i.e.*, affect enzymatic activity, binding activity, *etc.*) of a nGPCR-x polypeptide. HTS assays permit screening of large numbers of compounds in an efficient manner. Cell-based HTS systems are contemplated to investigate nGPCR-x receptor-ligand interaction. HTS assays are designed to identify "hits" or "lead compounds" having the desired property, from which modifications can be designed to improve the desired property. Chemical modification of the "hit" or "lead compound" is often based on an identifiable structure/activity relationship between the "hit" and the nGPCR-x polypeptide.

Another aspect of the present invention is directed to methods of identifying compounds which modulate (*i.e.*, increase or decrease) activity of nGPCR-x comprising

contacting nGPCR-x with a compound, and determining whether the compound modifies activity of nGPCR-x. The activity in the presence of the test compound is measured to the activity in the absence of the test compound. Where the activity of the sample containing the test compound is higher than the activity in the sample lacking the test compound, the compound will have increased activity. Similarly, where the activity of the sample containing the test compound is lower than the activity in the sample lacking the test compound, the compound will have inhibited activity.

The present invention is particularly useful for screening compounds by using nGPCR-x in any of a variety of drug screening techniques. The compounds to be screened include (which may include compounds which are suspected to modulate nGPCR-x activity), but are not limited to, extracellular, intracellular, biologic or chemical origin. The nGPCR-x polypeptide employed in such a test may be in any form, preferably, free in solution, attached to a solid support, borne on a cell surface or located intracellularly. One skilled in the art can, for example, measure the formation of complexes between nGPCR-x and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between nGPCR-x and its substrate caused by the compound being tested.

The activity of nGPCR-x polypeptides of the invention can be determined by, for example, examining the ability to bind or be activated by chemically synthesized peptide ligands. Alternatively, the activity of nGPCR-x polypeptides can be assayed by examining their ability to bind calcium ions, hormones, chemokines, neuropeptides, neurotransmitters, nucleotides, lipids, odorants, and photons. Alternatively, the activity of the nGPCR-x polypeptides can be determined by examining the activity of effector molecules including, but not limited to, adenylate cyclase, phospholipases and ion channels. Thus, modulators of nGPCR-x polypeptide activity may alter a GPCR receptor function, such as a binding property of a receptor or an activity such as G protein-mediated signal transduction or membrane localization. In various embodiments of the method, the assay may take the form of an ion flux assay, a yeast growth assay, a non-hydrolyzable GTP assay such as a [³⁵S]-GTP S assay, a cAMP assay, an inositol triphosphate assay, a diacylglycerol assay, an Aequorin assay, a Luciferase assay, a FLIPR assay for intracellular Ca²⁺ concentration, a mitogenesis assay, a MAP Kinase activity assay, an arachidonic acid release assay (e.g., using [³H]-arachidonic acid), and an assay for extracellular acidification rates, as well as other binding or function-based assays of nGPCR-x activity that are generally known in the art. In several of these embodiments, the invention comprehends the inclusion of any of the G proteins known in the art, such as G₁₆, G₁₅, or chimeric G_{qds}, G_{qs5}, G_{qo5}, G_{q25}, and the like. nGPCR-x

activity can be determined by methodologies that are used to assay for FaRP activity, which is well known to those skilled in the art. Biological activities of nGPCR-x receptors according to the invention include, but are not limited to, the binding of a natural or an unnatural ligand, as well as any one of the functional activities of GPCRs known in the art.

- 5 Non-limiting examples of GPCR activities include transmembrane signaling of various forms, which may involve G protein association and/or the exertion of an influence over G protein binding of various guanylate nucleotides; another exemplary activity of GPCRs is the binding of accessory proteins or polypeptides that differ from known G proteins.

The modulators of the invention exhibit a variety of chemical structures, which can be
10 generally grouped into non-peptide mimetics of natural GPCR receptor ligands, peptide and non-peptide allosteric effectors of GPCR receptors, and peptides that may function as activators or inhibitors (competitive, uncompetitive and non-competitive) (e.g., antibody products) of GPCR receptors. The invention does not restrict the sources for suitable modulators, which may be obtained from natural sources such as plant, animal or mineral
15 extracts, or non-natural sources such as small molecule libraries, including the products of combinatorial chemical approaches to library construction, and peptide libraries. Examples of peptide modulators of GPCR receptors exhibit the following primary structures: GLGPRPLRFamide, GNSFLRFamide, GGPQGPLRFamide, GPSGPLRFamide, PDVDHVFLRFamide, and pyro-EDVDHVFLRFamide.

- 20 Other assays can be used to examine enzymatic activity including, but not limited to, photometric, radiometric, HPLC, electrochemical, and the like, which are described in, for example, *Enzyme Assays: A Practical Approach*, eds. R. Eisenthal and M. J. Danson, 1992, Oxford University Press, which is incorporated herein by reference in its entirety.

The use of cDNAs encoding GPCRs in drug discovery programs is well-known;
25 assays capable of testing thousands of unknown compounds per day in high-throughput screens (HTSs) are thoroughly documented. The literature is replete with examples of the use of radiolabelled ligands in HTS binding assays for drug discovery (see Williams, *Medicinal Research Reviews*, 1991, 11, 147-184.; Sweetnam, *et al.*, *J. Natural Products*, 1993, 56, 441-455 for review). Recombinant receptors are preferred for binding assay HTS because they
30 allow for better specificity (higher relative purity), provide the ability to generate large amounts of receptor material, and can be used in a broad variety of formats (see Hodgson, *Bio/Technology*, 1992, 10, 973-980; each of which is incorporated herein by reference in its entirety).

A variety of heterologous systems is available for functional expression of recombinant receptors that are well known to those skilled in the art. Such systems include bacteria (Strosberg, *et al.*, *Trends in Pharmacological Sciences*, 1992, 13, 95-98), yeast (Pausch, *Trends in Biotechnology*, 1997, 15, 487-494), several kinds of insect cells (Vanden
5 Broeck, *Int. Rev. Cytology*, 1996, 164, 189-268), amphibian cells (Jayawickreme *et al.*, *Current Opinion in Biotechnology*, 1997, 8, 629-634) and several mammalian cell lines (CHO, HEK293, COS, etc.; see Gerhardt, *et al.*, *Eur. J. Pharmacology*, 1997, 334, 1-23). These examples do not preclude the use of other possible cell expression systems, including cell lines obtained from nematodes (PCT application WO 98/37177).

10 In preferred embodiments of the invention, methods of screening for compounds that modulate nGPCR-x activity comprise contacting test compounds with nGPCR-x and assaying for the presence of a complex between the compound and nGPCR-x. In such assays, the ligand is typically labeled. After suitable incubation, free ligand is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the
15 ability of the particular compound to bind to nGPCR-x.

It is well known that activation of heterologous receptors expressed in recombinant systems results in a variety of biological responses, which are mediated by G proteins expressed in the host cells. Occupation of a GPCR by an agonist results in exchange of bound GDP for GTP at a binding site on the G_α subunit; one can use a radioactive, non-
20 hydrolyzable derivative of GTP, $GTP\gamma[^{35}S]$, to measure binding of an agonist to the receptor (Sim *et al.*, *Neuroreport*, 1996, 7, 729-733). One can also use this binding to measure the ability of antagonists to bind to the receptor by decreasing binding of $GTP\gamma[^{35}S]$ in the presence of a known agonist. One could therefore construct a HTS based on $GTP\gamma[^{35}S]$ binding, though this is not the preferred method.

25 The G proteins required for functional expression of heterologous GPCRs can be native constituents of the host cell or can be introduced through well-known recombinant technology. The G proteins can be intact or chimeric. Often, a nearly universally competent G protein (e.g., $G_{\alpha 16}$) is used to couple any given receptor to a detectable response pathway. G protein activation results in the stimulation or inhibition of other native proteins, events
30 that can be linked to a measurable response.

Examples of such biological responses include, but are not limited to, the following: the ability to survive in the absence of a limiting nutrient in specifically engineered yeast cells (Pausch, *Trends in Biotechnology*, 1997, 15, 487-494); changes in intracellular Ca^{2+}

concentration as measured by fluorescent dyes (Murphy, *et al.*, *Cur. Opinion Drug Disc. Dev.*, 1998, 1, 192-199). Fluorescence changes can also be used to monitor ligand-induced changes in membrane potential or intracellular pH; an automated system suitable for HTS has been described for these purposes (Schroeder, *et al.*, *J. Biomolecular Screening*, 1996, 1, 75-80). Melanophores prepared from *Xenopus laevis* show a ligand-dependent change in pigment organization in response to heterologous GPCR activation; this response is adaptable to HTS formats (Jayawickreme *et al.*, *Cur. Opinion Biotechnology*, 1997, 8, 629-634). Assays are also available for the measurement of common second messengers, including cAMP, phosphoinositides and arachidonic acid, but these are not generally preferred for HTS.

Preferred methods of HTS employing these receptors include permanently transfected CHO cells, in which agonists and antagonists can be identified by the ability to specifically alter the binding of GTPγ[³⁵S] in membranes prepared from these cells. In another embodiment of the invention, permanently transfected CHO cells could be used for the preparation of membranes which contain significant amounts of the recombinant receptor proteins; these membrane preparations would then be used in receptor binding assays, employing the radiolabelled ligand specific for the particular receptor. Alternatively, a functional assay, such as fluorescent monitoring of ligand-induced changes in internal Ca²⁺ concentration or membrane potential in permanently transfected CHO cells containing each of these receptors individually or in combination would be preferred for HTS. Equally preferred would be an alternative type of mammalian cell, such as HEK293 or COS cells, in similar formats. More preferred would be permanently transfected insect cell lines, such as *Drosophila* S2 cells. Even more preferred would be recombinant yeast cells expressing the *Drosophila melanogaster* receptors in HTS formats well known to those skilled in the art (e.g., Pausch, *Trends in Biotechnology*, 1997, 15, 487-494).

The invention contemplates a multitude of assays to screen and identify inhibitors of ligand binding to nGPCR-x receptors. In one example, the nGPCR-x receptor is immobilized and interaction with a binding partner is assessed in the presence and absence of a candidate modulator such as an inhibitor compound. In another example, interaction between the nGPCR-x receptor and its binding partner is assessed in a solution assay, both in the presence and absence of a candidate inhibitor compound. In either assay, an inhibitor is identified as a compound that decreases binding between the nGPCR-x receptor and its binding partner. Another contemplated assay involves a variation of the dihybrid assay wherein an inhibitor of

protein/protein interactions is identified by detection of a positive signal in a transformed or transfected host cell, as described in PCT publication number WO 95/20652, published August 3, 1995.

Candidate modulators contemplated by the invention include compounds selected from libraries of either potential activators or potential inhibitors. There are a number of different libraries used for the identification of small molecule modulators, including: (1) chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules. Chemical libraries consist of random chemical structures, some of which are analogs of known compounds or analogs of compounds that have been identified as "hits" or "leads" in other drug discovery screens, some of which are derived from natural products, and some of which arise from non-directed synthetic organic chemistry. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of plants or marine organisms. Natural product libraries include polyketides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. These libraries are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are non-peptide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997). Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to modulate activity.

Still other candidate inhibitors contemplated by the invention can be designed and include soluble forms of binding partners, as well as such binding partners as chimeric, or fusion, proteins. A "binding partner" as used herein broadly encompasses non-peptide modulators, as well as such peptide modulators as neuropeptides other than natural ligands, antibodies, antibody fragments, and modified compounds comprising antibody domains that are immunospecific for the expression product of the identified nGPCR-x gene.

The polypeptides of the invention are employed as a research tool for identification, characterization and purification of interacting, regulatory proteins. Appropriate labels are

incorporated into the polypeptides of the invention by various methods known in the art and the polypeptides are used to capture interacting molecules. For example, molecules are incubated with the labeled polypeptides, washed to remove unbound polypeptides, and the polypeptide complex is quantified. Data obtained using different concentrations of polypeptide are used to calculate values for the number, affinity, and association of polypeptide with the protein complex.

Labeled polypeptides are also useful as reagents for the purification of molecules with which the polypeptide interacts including, but not limited to, inhibitors. In one embodiment of affinity purification, a polypeptide is covalently coupled to a chromatography column. Cells and their membranes are extracted, and various cellular subcomponents are passed over the column. Molecules bind to the column by virtue of their affinity to the polypeptide. The polypeptide-complex is recovered from the column, dissociated and the recovered molecule is subjected to protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotides for cloning the corresponding gene from an appropriate cDNA library.

Alternatively, compounds may be identified which exhibit similar properties to the ligand for the nGPCR-x of the invention, but which are smaller and exhibit a longer half time than the endogenous ligand in a human or animal body. When an organic compound is designed, a molecule according to the invention is used as a "lead" compound. The design of mimetics to known pharmaceutically active compounds is a well-known approach in the development of pharmaceuticals based on such "lead" compounds. Mimetic design, synthesis and testing are generally used to avoid randomly screening a large number of molecules for a target property. Furthermore, structural data deriving from the analysis of the deduced amino acid sequences encoded by the DNAs of the present invention are useful to design new drugs, more specific and therefore with a higher pharmacological potency.

Comparison of the protein sequence of the present invention with the sequences present in all the available databases showed a significant homology with the transmembrane portion of G protein coupled receptors. Accordingly, computer modeling can be used to develop a putative tertiary structure of the proteins of the invention based on the available information of the transmembrane domain of other proteins. Thus, novel ligands based on the predicted structure of nGPCR-x can be designed.

In a particular embodiment, the novel molecules identified by the screening methods according to the invention are low molecular weight organic molecules, in which case a composition or pharmaceutical composition can be prepared thereof for oral intake, such as in

tablets. The compositions, or pharmaceutical compositions, comprising the nucleic acid molecules, vectors, polypeptides, antibodies and compounds identified by the screening methods described herein, can be prepared for any route of administration including, but not limited to, oral, intravenous, cutaneous, subcutaneous, nasal, intramuscular or intraperitoneal.

5 The nature of the carrier or other ingredients will depend on the specific route of administration and particular embodiment of the invention to be administered. Examples of techniques and protocols that are useful in this context are, *inter alia*, found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A (ed.), 1980, which is incorporated herein by reference in its entirety.

10 The dosage of these low molecular weight compounds will depend on the disease state or condition to be treated and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. For treating human or animals, between approximately 0.5 mg/kg of body weight to 500 mg/kg of body weight of the compound can be administered. Therapy is typically administered at lower dosages and
15 is continued until the desired therapeutic outcome is observed.

The present compounds and methods, including nucleic acid molecules, polypeptides, antibodies, compounds identified by the screening methods described herein, have a variety of pharmaceutical applications and may be used, for example, to treat or prevent unregulated cellular growth, such as cancer cell and tumor growth. In a particular embodiment, the
20 present molecules are used in gene therapy. For a review of gene therapy procedures, see e.g. Anderson, *Science*, 1992, 256, 808-813, which is incorporated herein by reference in its entirety.

The present invention also encompasses a method of agonizing (stimulating) or antagonizing a nGPCR-x natural binding partner associated activity in a mammal comprising
25 administering to said mammal an agonist or antagonist to one of the above disclosed polypeptides in an amount sufficient to effect said agonism or antagonism. One embodiment of the present invention, then, is a method of treating diseases in a mammal with an agonist or antagonist of the protein of the present invention comprises administering the agonist or antagonist to a mammal in an amount sufficient to agonize or antagonize nGPCR-x-
30 associated functions.

In an effort to discover novel treatments for diseases, biomedical researchers and chemists have designed, synthesized, and tested molecules that inhibit the function of protein polypeptides. Some small organic molecules form a class of compounds that modulate the function of protein polypeptides. Examples of molecules that have been reported to inhibit

the function of protein kinases include, but are not limited to, bis monocyclic, bicyclic or heterocyclic aryl compounds (PCT WO 92/20642, published November 26, 1992 by Maguire *et al.*), vinylene-azaindole derivatives (PCT WO 94/14808, published July 7, 1994 by Ballinari *et al.*), 1-cyclopropyl-4-pyridyl-quinolones (U.S. Patent No. 5,330,992), styryl compounds (U.S. Patent No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), certain quinazoline derivatives (EP Application No. 0 566 266 A1), seleoindoles and selenides (PCT WO 94/03427, published February 17, 1994 by Denny *et al.*), tricyclic polyhydroxylic compounds (PCT WO 92/21660, published December 10, 1992 by Dow), and benzylphosphonic acid compounds (PCT WO 91/15495, published October 17, 1991 by Dow *et al.*), all of which are incorporated by reference herein, including any drawings.

Exemplary diseases and conditions amenable to treatment based on the present invention include, but are not limited to, thyroid disorders (*e.g.* thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Chron's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder, post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, *etc.*); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); sexual dysfunction, among others.

Compounds that can traverse cell membranes and are resistant to acid hydrolysis are potentially advantageous as therapeutics as they can become highly bioavailable after being administered orally to patients. However, many of these protein inhibitors only weakly inhibit function. In addition, many inhibit a variety of protein kinases and will therefore cause multiple side effects as therapeutics for diseases.

Some indolinone compounds, however, form classes of acid resistant and membrane permeable organic molecules. WO 96/22976 (published August 1, 1996 by Ballinari *et al.*) describes hydrosoluble indolinone compounds that harbor tetralin, naphthalene, quinoline, and indole substituents fused to the oxindole ring. These bicyclic substituents are in turn substituted with polar groups including hydroxylated alkyl, phosphate, and ether substituents. U.S. Patent Application Serial Nos. 08/702,232, filed August 23, 1996, entitled "Indolinone Combinatorial Libraries and Related Products and Methods for the Treatment of Disease" by Tang *et al.* (Lyon & Lyon Docket No. 221/187) and 08/485,323, filed June 7, 1995, entitled "Benzylidene-Z-Indoline Compounds for the Treatment of Disease" by Tang *et al.* (Lyon & Lyon Docket No. 223/298) and International Patent Publication WO 96/22976, published August 1, 1996 by Ballinari *et al.*, all of which are incorporated herein by reference in their entirety, including any drawings, describe indolinone chemical libraries of indolinone compounds harboring other bicyclic moieties as well as monocyclic moieties fused to the oxindole ring. Applications 08/702,232, filed August 23, 1996, entitled "Indolinone Combinatorial Libraries and Related Products and Methods for the Treatment of Disease" by Tang *et al.* (Lyon & Lyon Docket No. 221/187), 08/485,323, filed June 7, 1995, entitled "Benzylidene-Z-Indoline Compounds for the Treatment of Disease" by Tang *et al.* (Lyon & Lyon Docket No. 223/298), and WO 96/22976, published August 1, 1996 by Ballinari *et al.* teach methods of indolinone synthesis, methods of testing the biological activity of indolinone compounds in cells, and inhibition patterns of indolinone derivatives, both of which are incorporated by reference herein, including any drawings.

Other examples of substances capable of modulating kinase activity include, but are not limited to, tyrphostins, quinazolines, quinoxolines, and quinolines. The quinazolines, tyrphostins, quinolines, and quinoxolines referred to above include well-known compounds such as those described in the literature. For example, representative publications describing quinazolines include Barker *et al.*, EPO Publication No. 0 520 722 A1; Jones *et al.*, U.S. Patent No. 4,447,608; Kabbe *et al.*, U.S. Patent No. 4,757,072; Kaul and Vougioukas, U.S. Patent No. 5, 316,553; Kreighbaum and Comer, U.S. Patent No. 4,343,940; Pegg and Wardleworth, EPO Publication No. 0 562 734 A1; Barker *et al.*, Proc. of Am. Assoc. for Cancer Research 32:327 (1991); Bertino, J.R., Cancer Research 3:293-304 (1979); Bertino, J.R., Cancer Research 9(2 part 1):293-304 (1979); Curtin *et al.*, Br. J. Cancer 53:361-368 (1986); Fernandes *et al.*, Cancer Research 43:1117-1123 (1983); Ferris *et al.* J. Org. Chem. 44(2):173-178; Fry *et al.*, Science 265:1093-1095 (1994); Jackman *et al.*, Cancer Research 51:5579-5586 (1981); Jones *et al.* J. Med. Chem. 29(6):1114-1118; Lee and Skibo,

Biochemistry 26(23):7355-7362 (1987); Lemus *et al.*, J. Org. Chem. 54:3511-3518 (1989); Ley and Seng, Synthesis 1975:415-522 (1975); Maxwell *et al.*, Magnetic Resonance in Medicine 17:189-196 (1991); Mini *et al.*, Cancer Research 45:325-330 (1985); Phillips and Castle, J. Heterocyclic Chem. 17(19):1489-1596 (1980); Reece *et al.*, Cancer Research 5 47(11):2996-2999 (1977); Sculier *et al.*, Cancer Immunol. and Immunother. 23:A65 (1986); Sikora *et al.*, Cancer Letters 23:289-295 (1984); and Sikora *et al.*, Analytical Biochem. 172:344-355 (1988), all of which are incorporated herein by reference in their entirety, including any drawings.

Quinoxaline is described in Kaul and Vougioukas, U.S. Patent No. 5,316,553, 10 incorporated herein by reference in its entirety, including any drawings.

Quinolines are described in Dolle *et al.*, J. Med. Chem. 37:2627-2629 (1994); MaGuire, J. Med. Chem. 37:2129-2131 (1994); Burke *et al.*, J. Med. Chem. 36:425-432 (1993); and Burke *et al.* BioOrganic Med. Chem. Letters 2:1771-1774 (1992); all of which are incorporated by reference in their entirety, including any drawings.

15 Tyrophostins are described in Allen *et al.*, Clin. Exp. Immunol. 91:141-156 (1993); Anafi *et al.*, Blood 82:12:3524-3529 (1993); Baker *et al.*, J. Cell Sci. 102:543-555 (1992); Bilder *et al.*, Amer. Physiol. Soc. pp. 6363-6143:C721-C730 (1991); Brunton *et al.*, Proceedings of Amer. Assoc. Cancer Rsch. 33:558 (1992); Bryckaert *et al.*, Experimental Cell Research 199:255-261 (1992); Dong *et al.*, J. Leukocyte Biology 53:53-60 (1993); Dong 20 *et al.*, J. Immunol. 151(5):2717-2724 (1993); Gazit *et al.*, J. Med. Chem. 32:2344-2352 (1989); Gazit *et al.*, J. Med. Chem. 36:3556-3564 (1993); Kaur *et al.*, Anti-Cancer Drugs 5:213-222 (1994); King *et al.*, Biochem. J. 275:413-418 (1991); Kuo *et al.*, Cancer Letters 74:197-202 (1993); Levitzki, A., The FASEB J. 6:3275-3282 (1992); Lyall *et al.*, J. Biol. Chem. 264:14503-14509 (1989); Peterson *et al.*, The Prostate 22:335-345 (1993); Pillemer *et al.*, Int. J. Cancer 50:80-85 (1992); Posner *et al.*, Molecular Pharmacology 45:673-683 25 (1993); Rendu *et al.*, Biol. Pharmacology 44(5):881-888 (1992); Sauro and Thomas, Life Sciences 53:371-376 (1993); Sauro and Thomas, J. Pharm. and Experimental Therapeutics 267(3):119-1125 (1993); Wolbring *et al.*, J. Biol. Chem. 269(36):22470-22472 (1994); and Yoneda *et al.*, Cancer Research 51:4430-4435 (1991); all of which are incorporated herein by 30 reference in their entirety, including any drawings.

Other compounds that could be used as modulators include oxindolinones such as those described in U.S. patent application Serial No. 08/702,232 filed August 23, 1996, incorporated herein by reference in its entirety, including any drawings.

Methods of determining the dosages of compounds to be administered to a patient and modes of administering compounds to an organism are disclosed in U.S. Application Serial No. 08/702,282, filed August 23, 1996 and International patent publication number WO 96/22976, published August 1 1996, both of which are incorporated herein by reference in
5 their entirety, including any drawings, figures or tables. Those skilled in the art will appreciate that such descriptions are applicable to the present invention and can be easily adapted to it.

The proper dosage depends on various factors such as the type of disease being treated, the particular composition being used and the size and physiological condition of the
10 patient. Therapeutically effective doses for the compounds described herein can be estimated initially from cell culture and animal models. For example, a dose can be formulated in animal models to achieve a circulating concentration range that initially takes into account the IC_{50} as determined in cell culture assays. The animal model data can be used to more accurately determine useful doses in humans.

15 Plasma half-life and biodistribution of the drug and metabolites in the plasma, tumors and major organs can also be determined to facilitate the selection of drugs most appropriate to inhibit a disorder. Such measurements can be carried out. For example, HPLC analysis can be performed on the plasma of animals treated with the drug and the location of radiolabeled compounds can be determined using detection methods such as X-ray, CAT
20 scan and MRI. Compounds that show potent inhibitory activity in the screening assays, but have poor pharmacokinetic characteristics, can be optimized by altering the chemical structure and retesting. In this regard, compounds displaying good pharmacokinetic characteristics can be used as a model.

Toxicity studies can also be carried out by measuring the blood cell composition. For
25 example, toxicity studies can be carried out in a suitable animal model as follows: 1) the compound is administered to mice (an untreated control mouse should also be used); 2) blood samples are periodically obtained via the tail vein from one mouse in each treatment group; and 3) the samples are analyzed for red and white blood cell counts, blood cell composition and the percent of lymphocytes versus polymorphonuclear cells. A comparison of results for
30 each dosing regime with the controls indicates if toxicity is present.

At the termination of each toxicity study, further studies can be carried out by sacrificing the animals (preferably, in accordance with the American Veterinary Medical Association guidelines Report of the American Veterinary Medical Assoc. Panel on Euthanasia, Journal of American Veterinary Medical Assoc., 202:229-249, 1993).

Representative animals from each treatment group can then be examined by gross necropsy for immediate evidence of metastasis, unusual illness or toxicity. Gross abnormalities in tissue are noted and tissues are examined histologically. Compounds causing a reduction in body weight or blood components are less preferred, as are compounds having an adverse effect on major organs. In general, the greater the adverse effect the less preferred the compound.

For the treatment of cancers the expected daily dose of a hydrophobic pharmaceutical agent is between 1 to 500 mg/day, preferably 1 to 250 mg/day, and most preferably 1 to 50 mg/day. Drugs can be delivered less frequently provided plasma levels of the active moiety are sufficient to maintain therapeutic effectiveness. Plasma levels should reflect the potency of the drug. Generally, the more potent the compound the lower the plasma levels necessary to achieve efficacy.

nGPCR-x mRNA transcripts may found in many tissues, including, but not limited to, brain, peripheral blood lymphocytes, pancreas, ovary, uterus, testis, salivary gland, kidney, adrenal gland, liver, bone marrow, prostate, fetal liver, colon, muscle, and fetal brain, and may be found in many other tissues. Within the brain, nGPCR-x mRNA transcripts may be found in many tissues, including, but not limited to, frontal lobe, hypothalamus, pons, cerebellum, caudate nucleus, and medulla. Tissues and brain regions where specific nGPCR mRNA transcripts are expressed are identified in the Examples, below.

Odd numbered nucleotide sequences ranging from SEQ ID NO: 1 to SEQ ID NO: 93, SEQ ID NO: 185 and SEQ ID NO: 191 will, as detailed above, enable screening the endogenous neurotransmitters/hormones/ligands which activate, agonize, or antagonize nGPCR-x and for compounds with potential utility in treating disorders including, but not limited to, thyroid disorders (e.g. thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (e.g., Chron's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (e.g., pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder, post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, etc.); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (e.g., type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial

infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); sexual dysfunction, among others.

5 For example, nGPCR-x may be useful in the treatment of respiratory ailments such as asthma, where T cells are implicated by the disease. Contraction of airway smooth muscle is stimulated by thrombin. Cicala *et al* (1999) Br J Pharmacol 126:478-484. Additionally, in bronchiolitis obliterans, it has been noted that activation of thrombin receptors may be deleterious. Hauck *et al.* (1999) Am J Physiol 277:L22-L29. Furthermore, mast cells have
10 also been shown to have thrombin receptors. Cirino *et al* (1996) J Exp Med 183:821-827. nGPCR-x may also be useful in remodeling of airway structures in chronic pulmonary inflammation via stimulation of fibroblast procollagen synthesis. See, *e.g.*, Chambers *et al.* (1998) Biochem J 333:121-127; Trejo *et al.* (1996) J Biol Chem 271:21536-21541.

In another example, increased release of sCD40L and expression of CD40L by T cells
15 after activation of thrombin receptors suggests that nGPCR-x may be useful in the treatment of unstable angina due to the role of T cells and inflammation. See Aukrust *et al.* (1999) Circulation 100:614-620.

A further example is the treatment of inflammatory diseases, such as psoriasis, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and thyroiditis. Due to
20 the tissue expression profile of nGPCR-x, inhibition of thrombin receptors may be beneficial for these diseases. See, *e.g.*, Morris *et al.* (1996) Ann Rheum Dis 55:841-843. In addition to T cells, NK cells and monocytes are also critical cell types which contribute to the pathogenesis of these diseases. See, *e.g.*, Naldini & Carney (1996) Cell Immunol 172:35-42; Hoffman & Cooper (1995) Blood Cells Mol Dis 21:156-167; Colotta *et al.* (1994) Am J
25 Pathol 144:975-985.

Expression of nGPCR-x in bone marrow and spleen may suggest that it may play a role in the proliferation of hematopoietic progenitor cells. See DiCuccio *et al.* (1996) Exp Hematol 24:914-918.

As another example, nGPCR-x may be useful in the treatment of acute and/or
30 traumatic brain injury. Astrocytes have been demonstrated to express thrombin receptors. Activation of thrombin receptors may be involved in astrogliosis following brain injury. Therefore, inhibition of receptor activity may be beneficial for limiting neuroinflammation. Scar formation mediated by astrocytes may also be limited by inhibiting thrombin receptors.

See, e.g., Pindon *et al.* (1998) *Eur J Biochem* 255:766-774; Uhl & Reiser. (1997) *Glia* 21:361-369; Grabham & Cunningham (1995) *J Neurochem* 64:583-591.

nGPCR-x receptor activation may mediate neuronal and astrocyte apoptosis and prevention of neurite outgrowth. Inhibition would be beneficial in both chronic and acute
 5 brain injury. See, e.g., Donovan *et al.* (1997) *J Neurosci* 17:5316-5326; Turgeon *et al.* (1998) *J Neurosci* 18:6882-6891; Smith-Swintosky *et al.* (1997) *J Neurochem* 69:1890-1896; Gill *et al.* (1998) *Brain Res* 797:321-327; Suidan *et al.* (1996) *Semin Thromb Hemost* 22:125-133.

The attached Sequence Listing contains the sequences of the polynucleotides and polypeptides of the invention and is incorporated herein by reference in its entirety.

10 As described above and in Example 4 below, the genes encoding nGPCR-1 (nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 73, amino acid sequence SEQ ID NO: 2, SEQ ID NO:74), nGPCR-9 (nucleic acid sequence SEQ ID NO:9, SEQ ID NO:77, amino acid sequence SEQ ID NO:10, SEQ ID NO:78), nGPCR-11 (nucleic acid sequence SEQ ID NO:11, SEQ ID NO:79, amino acid sequence SEQ ID NO:12, SEQ ID NO:80), nGPCR-16
 15 (nucleic acid sequence SEQ ID NO: 21, SEQ ID NO:81, amino acid sequence SEQ ID NO: 22, SEQ ID NO:82), nGPCR-40 (nucleic acid sequence SEQ ID NO:53, SEQ ID NO:83, amino acid sequence SEQ ID NO:54, SEQ ID NO:84), nGPCR-54 (nucleic acid sequence SEQ ID NO:59, SEQ ID NO:85, amino acid sequence SEQ ID NO:60, SEQ ID NO: 86), nGPCR-56 (nucleic acid sequence SEQ ID NO:63, SEQ ID NO:87, SEQ ID NO:89, amino
 20 acid sequence SEQ ID NO:64, SEQ ID NO: 88, SEQ ID NO:90), nGPCR-58 (nucleic acid sequence SEQ ID NO:3, SEQ ID NO:185, amino acid sequence SEQ ID NO:4, SEQ ID NO: 186) have been detected in brain tissue indicating that these n-GPCR-x proteins are neuroreceptors. The identification of modulators such as agonists and antagonists is therefore useful for the identification of compounds useful to treat neurological diseases and disorders.
 25 Such neurological diseases and disorders, including but are not limited to, schizophrenia, affective disorders, ADHD/ADD (*i.e.*, Attention Deficit-Hyperactivity Disorder/Attention Deficit Disorder), and neural disorders such as Alzheimer's disease, Parkinson's disease, migraine, and senile dementia as well as depression, anxiety, bipolar disease, epilepsy, neuritis, neurasthenia, neuropathy, neuroses, and the like.

30 **Methods of Screening Human Subjects**

Thus in yet another embodiment, the invention provides genetic screening procedures that entail analyzing a person's genome -- in particular their alleles for GPCRs of the invention -- to determine whether the individual possesses a genetic characteristic found in other individuals that are considered to be afflicted with, or at risk for, developing a mental

disorder or disease of the brain that is suspected of having a hereditary component. For example, in one embodiment, the invention provides a method for determining a potential for developing a disorder affecting the brain in a human subject comprising the steps of analyzing the coding sequence of one or more GPCR genes from the human subject; and
5 determining development potential for the disorder in said human subject from the analyzing step.

More particularly, the invention provides a method of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor, comprising the steps of: (a) assaying nucleic acid of a human subject to determine a presence or an absence
10 of a mutation altering the amino acid sequence, expression, or biological activity of at least one seven transmembrane receptor that is expressed in the brain, wherein the seven transmembrane receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 74, 186, 78, 80, 82, 84, 86, 90, and 94, or an allelic variant thereof, and wherein the nucleic acid corresponds to the gene encoding the seven
15 transmembrane receptor; and (b) diagnosing the disorder or predisposition from the presence or absence of said mutation, wherein the presence of a mutation altering the amino acid sequence, expression, or biological activity of allele in the nucleic acid correlates with an increased risk of developing the disorder. In preferred variations, the seven transmembrane receptor is nGPCR-40 or nGPCR-54 comprising amino acid sequences set forth in SEQ ID
20 NO: 84 for nGPCR-40 and SEQ ID NO: 86 for nGPCR-54, or an allelic variant thereof, and the disease is schizophrenia.

By "human subject" is meant any human being, human embryo, or human fetus. It will be apparent that methods of the present invention will be of particular interest to individuals that have themselves been diagnosed with a disorder affecting the brain or have
25 relatives that have been diagnosed with a disorder affecting the brain.

By "screening for an increased risk" is meant determination of whether a genetic variation exists in the human subject that correlates with a greater likelihood of developing a disorder affecting the brain than exists for the human population as a whole, or for a relevant racial or ethnic human sub-population to which the individual belongs. Both positive and
30 negative determinations (i.e., determinations that a genetic predisposition marker is present or is absent) are intended to fall within the scope of screening methods of the invention. In preferred embodiments, the presence of a mutation altering the sequence or expression of at least one nGPCR-40 or nGPCR-54 seven transmembrane receptor allele in the nucleic acid is

correlated with an increased risk of developing schizophrenia, whereas the absence of such a mutation is reported as a negative determination.

The "assaying" step of the invention may involve any techniques available for analyzing nucleic acid to determine its characteristics, including but not limited to well-known techniques such as single-strand conformation polymorphism analysis (SSCP) [Orita *et al.*, *Proc Natl. Acad. Sci. USA*, 86: 2766-2770 (1989)]; heteroduplex analysis [Whiteet *et al.*, *Genomics*, 12: 301-306 (1992)]; denaturing gradient gel electrophoresis analysis [Fischer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80: 1579-1583 (1983); and Riesner *et al.*, *Electrophoresis*, 10: 377-389 (1989)]; DNA sequencing; RNase cleavage [Myers *et al.*, *Science*, 230: 1242-1246 (1985)]; chemical cleavage of mismatch techniques [Rowley *et al.*, *Genomics*, 30: 574-582 (1995); and Roberts *et al.*, *Nucl. Acids Res.*, 25: 3377-3378 (1997)]; restriction fragment length polymorphism analysis; single nucleotide primer extension analysis [Shumaker *et al.*, *Hum. Mutat.*, 7: 346-354 (1996); and Pastinen *et al.*, *Genome Res.*, 7: 606-614 (1997)]; 5' nuclease assays [Pease *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:5022-5026 (1994)]; DNA Microchip analysis [Ramsay, G., *Nature Biotechnology*, 16: 40-48 (1999); and Chee *et al.*, U.S. Patent No. 5,837,832]; and ligase chain reaction [Whiteley *et al.*, U.S. Patent No. 5,521,065]. [See generally, Schafer and Hawkins, *Nature Biotechnology*, 16: 33-39 (1998).] All of the foregoing documents are hereby incorporated by reference in their entirety.

Thus, in one preferred embodiment involving screening nGPCR-40 or nGPCR-54 sequences, for example, the assaying step comprises at least one procedure selected from the group consisting of: (a) determining a nucleotide sequence of at least one codon of at least one nGPCR-40 or nGPCR-54 allele of the human subject; (b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; (c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and (d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences.

In a highly preferred embodiment, the assaying involves sequencing of nucleic acid to determine nucleotide sequence thereof, using any available sequencing technique. [See, *e.g.*, Sanger *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 74: 5463-5467 (1977) (dideoxy chain termination method); Mirzabekov, *TIBTECH*, 12: 27-32 (1994) (sequencing by hybridization); Drmanac *et al.*, *Nature Biotechnology*, 16: 54-58 (1998); U.S. Patent No. 5,202,231; and *Science*, 260:

1649-1652 (1993) (sequencing by hybridization); Kieleczawa *et al.*, *Science*, 258: 1787-1791 (1992) (sequencing by primer walking); (Douglas *et al.*, *Biotechniques*, 14: 824-828 (1993) (Direct sequencing of PCR products); and Akane *et al.*, *Biotechniques* 16: 238-241 (1994); Maxam and Gilbert, *Meth. Enzymol.*, 65: 499-560 (1977) (chemical termination sequencing),
5 all incorporated herein by reference.] The analysis may entail sequencing of the entire nGPCR gene genomic DNA sequence, or portions thereof; or sequencing of the entire seven transmembrane receptor coding sequence or portions thereof. In some circumstances, the analysis may involve a determination of whether an individual possesses a particular allelic variant, in which case sequencing of only a small portion of nucleic acid-- enough to
10 determine the sequence of a particular codon characterizing the allelic variant-- is sufficient. This approach is appropriate, for example, when assaying to determine whether one family member inherited the same allelic variant that has been previously characterized for another family member, or, more generally, whether a person's genome contains an allelic variant that has been previously characterized and correlated with a mental disorder having a heritable
15 component.

In another highly preferred embodiment, the assaying step comprises performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences. In a preferred embodiment, the hybridization involves a determination of whether nucleic acid
20 derived from the human subject will hybridize with one or more oligonucleotides, wherein the oligonucleotides have nucleotide sequences that correspond identically to a portion of the GPCR gene sequence taught herein, such as the nGPCR-40 or nGPCR-54 coding sequence set forth in SEQ ID NOS: 83 for nGPCR-40 or 85 for nGPCR-54, or that correspond identically except for one mismatch. The hybridization conditions are selected to
25 differentiate between perfect sequence complementarity and imperfect matches differing by one or more bases. Such hybridization experiments thereby can provide single nucleotide polymorphism sequence information about the nucleic acid from the human subject, by virtue of knowing the sequences of the oligonucleotides used in the experiments.

Several of the techniques outlined above involve an analysis wherein one performs a
30 polynucleotide migration assay, *e.g.*, on a polyacrylamide electrophoresis gel (or in a capillary electrophoresis system), under denaturing or non-denaturing conditions. Nucleic acid derived from the human subject is subjected to gel electrophoresis, usually adjacent to (or co-loaded with) one or more reference nucleic acids, such as reference GPCR-encoding sequences having a coding sequence identical to all or a portion of SEQ ID NOS: 83 or 85 (or

identical except for one known polymorphism). The nucleic acid from the human subject and the reference sequence(s) are subjected to similar chemical or enzymatic treatments and then electrophoresed under conditions whereby the polynucleotides will show a differential migration pattern, unless they contain identical sequences. [See generally Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, New York: John Wiley & Sons, Inc. (1987-1999); and Sambrook *et al.*, (eds.), *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press (1989), both incorporated herein by reference in their entirety.]

In the context of assaying, the term "nucleic acid of a human subject" is intended to include nucleic acid obtained directly from the human subject (*e.g.*, DNA or RNA obtained from a biological sample such as a blood, tissue, or other cell or fluid sample); and also nucleic acid derived from nucleic acid obtained directly from the human subject. By way of non-limiting examples, well known procedures exist for creating cDNA that is complementary to RNA derived from a biological sample from a human subject, and for amplifying (*e.g.*, via polymerase chain reaction (PCR)) DNA or RNA derived from a biological sample obtained from a human subject. Any such derived polynucleotide which retains relevant nucleotide sequence information of the human subject's own DNA/RNA is intended to fall within the definition of "nucleic acid of a human subject" for the purposes of the present invention.

In the context of assaying, the term "mutation" includes addition, deletion, and/or substitution of one or more nucleotides in the GPCR gene sequence (*e.g.*, as compared to the seven transmembrane receptor-encoding sequences set forth of SEQ ID NOS: 74, 186, 78, 80, 82, 84, 86, 90, and 94) and other polymorphisms that occur in introns (where introns exist) and that are identifiable via sequencing, restriction fragment length polymorphism, or other techniques. The various activity examples provided herein permit determination of whether a mutation modulates activity of the relevant receptor in the presence or absence of various test substances.

In a related embodiment, the invention provides methods of screening a person's genotype with respect to GPCR's of the invention, and correlating such genotypes with diagnoses for disease or with predisposition for disease (for genetic counseling). For example, the invention provides a method of screening for an nGPCR-40 or nGPCR-54 hereditary schizophrenia genotype in a human patient, comprising the steps of: (a) providing a biological sample comprising nucleic acid from the patient, the nucleic acid including sequences corresponding to said patient's nGPCR-40 or nGPCR-54 alleles; (b) analyzing the

nucleic acid for the presence of a mutation or mutations; (c) determining an nGPCR-40 or nGPCR-54 genotype from the analyzing step; and (d) correlating the presence of a mutation in an nGPCR-40 or nGPCR-54 allele with a hereditary schizophrenia genotype. In a preferred embodiment, the biological sample is a cell sample containing human cells that
5 contain genomic DNA of the human subject. The analyzing can be performed analogously to the assaying described in preceding paragraphs. For example, the analyzing comprises sequencing a portion of the nucleic acid (e.g., DNA or RNA), the portion comprising at least one codon of the nGPCR-40 or nGPCR-54 alleles.

Although more time consuming and expensive than methods involving nucleic acid
10 analysis, the invention also may be practiced by assaying protein of a human subject to determine the presence or absence of an amino acid sequence variation in GPCR protein from the human subject. Such protein analyses may be performed, e.g., by fragmenting GPCR protein via chemical or enzymatic methods and sequencing the resultant peptides; or by Western analyses using an antibody having specificity for a particular allelic variant of the
15 GPCR.

The invention also provides materials that are useful for performing methods of the invention. For example, the present invention provides oligonucleotides useful as probes in the many analyzing techniques described above. In general, such oligonucleotide probes comprise 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
20 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides that have a sequence that is identical, or exactly complementary, to a portion of a human GPCR gene sequence taught herein (or allelic variant thereof), or that is identical or exactly complementary except for one nucleotide substitution. In a preferred embodiment, the oligonucleotides have a sequence that corresponds in the foregoing manner to a human
25 GPCR coding sequence taught herein, and in particular, the coding sequences set forth in SEQ ID NO: 83 and 85. In one variation, an oligonucleotide probe of the invention is purified and isolated. In another variation, the oligonucleotide probe is labeled, e.g., with a radioisotope, chromophore, or fluorophore. In yet another variation, the probe is covalently attached to a solid support. [See generally Ausubel *et al.* And Sambrook *et al.*, *supra.*]

30 In a related embodiment, the invention provides kits comprising reagents that are useful for practicing methods of the invention. For example, the invention provides a kit for screening a human subject to diagnose schizophrenia or a genetic predisposition therefor, comprising, in association: (a) an oligonucleotide useful as a probe for identifying polymorphisms in a human nGPCR-40 or nGPCR-54 seven transmembrane receptor gene,

the oligonucleotide comprising 6-50 nucleotides that have a sequence that is identical or exactly complementary to a portion of a human nGPCR-40 or nGPCR-54 gene sequence or nGPCR-40 or nGPCR-54 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution;

5 and (b) a media packaged with the oligonucleotide containing information identifying polymorphisms identifiable with the probe that correlate with schizophrenia or a genetic predisposition therefor. Exemplary information-containing media include printed paper package inserts or packaging labels; and magnetic and optical storage media that are readable by computers or machines used by practitioners who perform genetic screening and

10 counseling services. The practitioner uses the information provided in the media to correlate the results of the analysis with the oligonucleotide with a diagnosis. In a preferred variation, the oligonucleotide is labeled.

In still another embodiment, the invention provides methods of identifying those allelic variants of GPCRs of the invention that correlate with mental disorders. For example,

15 the invention provides a method of identifying a seven transmembrane allelic variant that correlates with a mental disorder, comprising steps of: (a) providing a biological sample comprising nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny; (b) analyzing the nucleic acid for the presence of a mutation or mutations in at least one seven transmembrane receptor that is expressed in the

20 brain, wherein the at least one seven transmembrane receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 74, 186, 78, 80, 82, 84, 86, 90, and 94 or an allelic variant thereof, and wherein the nucleic acid includes sequence corresponding to the gene or genes encoding the at least one seven transmembrane receptor; (c) determining a genotype for the patient for the at least one seven transmembrane receptor

25 from said analyzing step; and (d) identifying an allelic variant that correlates with the mental disorder from the determining step. To expedite this process, it may be desirable to perform linkage studies in the patients (and possibly their families) to correlate chromosomal markers with disease states. The chromosomal localization data provided herein facilitates identifying an involved GPCR with a chromosomal marker.

30 The foregoing method can be performed to correlate GPCR's of the invention to a number of disorders having hereditary components that are causative or that predispose persons to the disorder. For example, in one preferred variation, the disorder is schizophrenia, and the at least one seven transmembrane receptor comprises nGPCR-40 having an amino acid sequence set forth in SEQ ID NO: 84 or an allelic variant thereof.

Also contemplated as part of the invention are polynucleotides that comprise the allelic variant sequences identified by such methods, and polypeptides encoded by the allelic variant sequences, and oligonucleotide and oligopeptide fragments thereof that embody the mutations that have been identified. Such materials are useful in *in vitro* cell-free and cell-based assays for identifying lead compounds and therapeutics for treatment of the disorders. For example, the variants are used in activity assays, binding assays, and assays to screen for activity modulators described herein. In one preferred embodiment, the invention provides a purified and isolated polynucleotide comprising a nucleotide sequence encoding a nGPCR-40 or nGPCR-54 receptor allelic variant identified according to the methods described above; and an oligonucleotide that comprises the sequences that differentiate the allelic variant from the nGPCR-40 or nGPCR-54 sequences set forth in SEQ ID NOS: 83 and 88. The invention also provides a vector comprising the polynucleotide (preferably an expression vector); and a host cell transformed or transfected with the polynucleotide or vector. The invention also provides an isolated cell line that is expressing the allelic variant GPCR polypeptide; purified cell membranes from such cells; purified polypeptide; and synthetic peptides that embody the allelic variation amino acid sequence. In one particular embodiment, the invention provides a purified polynucleotide comprising a nucleotide sequence encoding a nGPCR-40 seven transmembrane receptor protein of a human that is affected with schizophrenia; wherein said polynucleotide hybridizes to the complement of SEQ ID NO: 83 under the following hybridization conditions: (a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate and (b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS; and wherein the polynucleotide encodes a nGPCR-40 amino acid sequence that differs from SEQ ID NO: 84 by at least one residue.

An exemplary assay for using the allelic variants is a method for identifying a modulator of nGPCR-x biological activity, comprising the steps of: (a) contacting a cell expressing the allelic variant in the presence and in the absence of a putative modulator compound; (b) measuring nGPCR-x biological activity in the cell; and (c) identifying a putative modulator compound in view of decreased or increased nGPCR-x biological activity in the presence versus absence of the putative modulator.

Additional features of the invention will be apparent from the following Examples. Examples 1, 2, 4, 11, 12, and 13 are actual, while the remaining Examples are prophetic. Additional features and variations of the invention will be apparent to those skilled in the art

from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

10 EXAMPLES

EXAMPLE 1: IDENTIFICATION OF nGPCR-X

A. Database search

The Celera database was searched using known GPCR receptors as query sequences to find patterns suggestive of novel G protein-coupled receptors. Positive hits were further
15 analyzed with the GCG program BLAST to determine which ones were the most likely candidates to encode G protein-coupled receptors, using the standard (default) alignment produced by BLAST as a guide.

Briefly, the BLAST algorithm, which stands for Basic Local Alignment Search Tool is suitable for determining sequence similarity (Altschul *et al.*, J. Molec. Biol., 1990, 215, 403-410, which is incorporated herein by reference in its entirety). Software for performing
20 BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score
25 threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension for the word hits in each direction are halted when: 1) the cumulative alignment
30 score falls off by the quantity X from its maximum achieved value; 2) the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or 3) the end of either sequence is reached. The Blast algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The Blast program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff *et al.*, Proc.

Natl. Acad. Sci. USA, 1992, 89, 10915-10919, which is incorporated herein by reference in its entirety) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

5 The BLAST algorithm (Karlin *et al.*, Proc. Natl. Acad. Sci. USA, 1993, 90, 5873-5877, which is incorporated herein by reference in its entirety) and Gapped BLAST perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a
10 GPCR gene or cDNA if the smallest sum probability in comparison of the test nucleic acid to a GPCR nucleic acid is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Homology searches were performed with the program BLAST version 2.08. A collection of 340 query amino acid sequences derived from GPCR's was used to search the
15 genomic DNA sequence using TBLASTN and alignments with an E-value lower than 0.01 were collected from each BLAST search. The amino acid sequences have been edited to remove regions in the sequence that produce non-significant alignments with proteins that are not related to GPCR's.

Multiple query sequences may have a significant alignment to the same genomic
20 region, although each alignment may not cover exactly the same DNA region. A procedure is used to determine the region of maximum common overlap between the alignments from several query sequences. This region is called the consensus DNA region. The procedure for determining this consensus involves the automatic parsing of the BLAST output files using the program MSPcrunch to produce a tabular report. From this tabular report the start and
25 end of each alignment in the genomic DNA is extracted. This information was used by a PERL script to derive the maximum common overlap. These regions were reported in the form of a unique sequence identifier, a start and the end position in the sequence. The sequences defined by these regions were extracted from the original genomic sequence file using the program fetchdb.

30 The consensus regions were assembled into a non-redundant set by using the program phrap. After assembly with phrap a set of contigs and singletons was defined as candidate DNA regions coding for nGPCR-x. These sequences were then submitted for further sequence analysis.

Further sequence analysis involved the removal of sequences previously isolated and removal of sequences related to olfactory GPCRs. The transmembrane regions for the sequences that remained were determined using a FORTRAN computer program called "tmrest.all" [Parodi *et al.*, Comput.Appl.Biosci. 5:527-535(1994)]. Only sequences that
5 contained transmembrane regions in a pattern found in GPCRs were retained.

cDNAs were sequenced directly using an ABI377 fluorescence-based sequencer (Perkin-Elmer/Applied Biosystems Division, PE/ABD, Foster City, CA) and the ABI PRISMTM Ready Dye-Deoxy Terminator kit with Taq FSTM polymerase. Each ABI cycle sequencing reaction contained about 0.5 µg of plasmid DNA. Cycle-sequencing was
10 performed using an initial denaturation at 98°C for 1 minute, followed by 50 cycles using the following parameters: 98°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 60°C for 4 minutes. Temperature cycles and times were controlled by a Perkin-Elmer 9600 thermocycler. Extension products were purified using CentriflexTM gel filtration cartridges (Advanced Genetic Technologies Corp., Gaithersburg, MD). Each reaction
15 product was loaded by pipette onto the column, which is then centrifuged in a swinging bucket centrifuge (Sorvall model RT6000B tabletop centrifuge) at 1500 x g for 4 minutes at room temperature. Column-purified samples were dried under vacuum for about 40 minutes and then dissolved in 5 µl of a DNA loading solution (83% deionized formamide, 8.3 mM EDTA, and 1.6 mg/ml Blue Dextran). The samples were then heated to 90°C for three
20 minutes and loaded into the gel sample wells for sequence analysis using the ABI377 sequencer. Sequence analysis was performed by importing ABI377 files into the Sequencer program (Gene Codes, Ann Arbor, MI). Generally, sequence reads of 700 bp were obtained. Potential sequencing errors were minimized by obtaining sequence information from both DNA strands and by re-sequencing difficult areas using primers annealing at different
25 locations until all sequencing ambiguities were removed.

The following Table 5 contains the sequences of the polynucleotides and polypeptides of the invention. Start and stop codons within the polynucleotide sequence are identified by boldface type. The transmembrane domains within the polypeptide sequence are identified by underlining.

30

ACTCCTCGGTGCTGTTTCAGGTGTTTCTGGAATGGATCTTCTAGTTTCTGCTGGTAGATCCAGGAAGCATTCTGAAGTTTTTCCATCCCTGA

The following amino acid sequence <SEQ ID NO. 18> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 17:

SGMEKLQNASWIYQQKLEDPFQKHLNSTEEYLAFLCGPRRSHFFLPVSVVYVPIFVVGVIIGNVLVCLVILQHQAMKTPNTYYLFSLAVSDLLVLLLGMPLEVYEMWRNYPFLFGPVGCYFKTALFETVCFASILSITTVSVERYVALHLPFRAKLQSTRRRRLRILGIVWGFVSLFSLPNTSIHGKIFHYFPNGSLVPGSATCTVIKPMWIYNFIIQVTSFLFYLLPMTVISVLYLMLALRVSIAGVAG

The following DNA sequence beGPCR-**seq18** <SEQ ID NO. 19> was identified in *H. sapiens*:

ATCAAGATGATTTTTGCTATCGTGCAAATTATTTGGATTTTCCAACCTCATCTGTAATCCCATTGTCTATGCATTTATGAATGAAAACCTCAAAAAAATGTTTGTCTGCAGTTTGTATTGCATAGTAAATAAAACCTTCTCTCCAGCACAAAGGCATGGAAATTCAGGAATTACAATGATGCGGAAGAAAGCAAAGTTTTCCCTCAGAGAGAATCCAGTG

The following amino acid sequence <SEQ ID NO. 20> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 19:

IKMIFAIVQIIGFSNSICNPIVYAFMNENFKKNVLSAVCYCIVNKTFSPAQRHGNSGITMMRKKAKFSLRENP

The following DNA sequence beGPCR-**seq16** <SEQ ID NO. 21> was identified in *H. sapiens*:

GCCACAGCATGCAGTTTTCTGTAGAATTCCACTTTGTCTTTGCACTTGAAGAAGATGAGGTATCTGGTGACCAGGATCACACATAGAAATAGGAACCGTGAGGTACATGTGGATGTGCAGCATGGCACTCACAAATTTGCAGAAAGGCAGCCCAACATCCAAGTCTTCTTGATGAGGTAGGTCAAGCGAAATGGCACTGTCAGCAGAAAAACGCTGTGGACCACCACCAAGTTAATGACCGCCATGGTGGTCACTGACCGGGTGTTTCAATTTTACCAGGAGGAAGAATGGAAATGACACCCACCAGCCCGCCAATAAGCACTATGAAGTAGAGGCTGATTAAGTGGGGTGTCCTATAGGATCGCAAGAGGAATTCCTGGAGGTATTGTGGCCAGGCATACTTGGGAAGTCACCTGGAGGAGAAAAGCACAGAGTAACAGTAC

The following amino acid sequence <SEQ ID NO. 22> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 21:

VSYSGAFSPPGDFPSMPGHNTSRNSSCDPIVTPHLISLYFIVLIGGLVGVISILFLLVKMNTSRVTTMAVINLVVHVSFLLTVPFRLTYLIKKTWMFGLPFCKFVSAMLHIHMYLTVPILCGDPGHQIPHLLQVQRQSGILQKTACCG

The following DNA sequence beGPCR-**seq17** <SEQ ID NO. 23> was identified in *H. sapiens*:

ACTGACCAAGGTCAGGGCATCGACTGAGGCTAGAAGGCCACAGGAAATGCCAGTCAAGGTGTTGGCGCCTGCAATCGCACCTACCACAACTTGACCGGGGGCAGGGGGGCAGGCCCGCCAGCGAACACGGTCAGCAGCACAGTCCATTGCAGAGCACGGAGAGCAACACGATGGCCACACGGCCAGGCGGATGCCCCAGCTTTCAAAGAGTACTACA

The following amino acid sequence <SEQ ID NO. 24> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 23:

CEYLFESWGIRLAVWAIVLLSVLCNGLVLLTVFAGGPAPLPPPVKFVVGAIAGANTLTGISCGLLASVDALTLVS

The following DNA sequence beGPCR-**seq20** <SEQ ID NO. 25> was identified in *H. sapiens*:

AACCCCATCATCTACACGCTACCAACCGCAGCTGCGCCACGCGCTCCTGCGCCTGGTCTGCTGCGGACGCCACTCCTGCGGCAGAGACCCGAGTGCTCCAGCAGTCGGCAGCGCGGCTGAGGCTTCCGGGGGCTGC

CATCGTGCTAGAGAACTAGCCGTGTTGTTGGTGCTCGGACGCCACCCGCGCTTCCACGCTCCCATGTTCTGT
 CTCTGGGCAGCCTCACGTTGTGCGGATCTGCTGGCAGGCGCGCCCTACGCCGCCAACATCCTACTGTGCGGGC
 CGCTCAGCTGAAACTGTCCCCGCGCTCTGTTTCGCACGGGAGGGAGGCGTCTTCGTGGCACTCACTGCGCT
 CGTGCTGAGCCTCTGGCCATCGCGCTGGAGCGCAGCCTCACCATGGCGCGCAGGGGGCCCGCGCCCGTCTCC
 AGTCGGGGGCGCACGCTGGCGATGGCAGCCGCGGCTGGGGCGTGTGCTGCTCCTCGGGCTCCTGCCAGCGC
 TGGGCTGGAATTGCTTGGGTGCGCTGGACGCTTGTCTCACTGTCTGCGGCTCTACGCCAAGGCCTACGTGCT
 CTTCTGCGTGCTCGCCTTCGTGGGCATCCTGGCCGCTATCTGTGCACTCTACGCGCGCATCTACTGCCAGGTA
 CGCGCCAACGCGCGGCGCCTGCCGGCAGGCGCGGGACTGCGGGGACCACCTCGACCCGGGCGCGTCCGAAGC
 CGCGCTCGCTGGCCTTGTGCGCACGCTCAGCGTGGTGCTCCTGGCCTTTGTGGCATGTTGGGGCCCCCTCTT
 CCTGCTGCTGTTGCTCGACGTGGCGTGCCCGCGCGCACCTGTCTGTACTCCTGCAGGCCGATCCCTTCCTG
 GGACTGGCCATGGCCAACCTCACTTCTGAACCCCATCATCTACACGCTCACCAACCGCGACCTGCGCCACGCGC
 TCCTGCGCCTGGTCTGCTGCGGACGCCACTCCTGCGGCAGAGACCCGAGTGGCTCCAGCAGTCCGGCAGCGC
 GGCTGAGGCTTCCGGGGGCTGCGCGCGCTGCTGCCCGGGGCTTGATGGGAGCTTCAGCGGCTCGGAGCGC
 TCATCGCCCCAGCGCAGCGGCTGGACACCGAGCGGCTCCACAGGCAGCCCCGGTGCACCCACAGCCGCCCGGA
 CTCTGGTATCAGAACCGGCTGCAGACTGA

The following amino acid sequence <SEQ ID NO. 78> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 77:

MESGLLRPAPVSEVIVLHYNVTGKLRGARYQPGAGLRADAVVCLAVCAFIVLENLAVLLVLGRHPRFHAPMFL
LLGSLTSLDLAGAAYAANILLSGPLTLKLSPALWFAREGGVFVALTASVLSLLAIALERSLTMARRGPAPVS
SRGRTLAMAAAANGVSLLLGLLPALGWNCRLRLDACSTVLPYAKAYVLFVCLAFVGLAALCALYARIYQV
RANARRLPARPGTAGTTSTRARRKPRSLALLRLSVLLAFVACWGFLFLLLLLDVACPARTCPVLLQADPFL
GLAMANSLLNPIIYTLTNRLRLHALLRLVCCGRHSCGRDPSGSQASAAEASGGLRRLCPGLDGSFSGSER
SSPQRDGLDTSGSTGSPGAPTAARTLVSEPAAD

The following DNA sequence nGPCR-11 <SEQ ID NO. 79> was identified in *H. sapiens*:

ATGTACAACGGGTGCTGCTGCCGCATCGAGGGGGACACCATCTCCAGGTGATGCCGCCGTGCTCATTTGTGG
 CCTTTGTGCTGGGCGCACTAGGCAATGGGGTTCGCCCTGTGTGGTTTCTGCTTCCACATGAAGACCTGGAAGCC
 CAGCACTGTTTACCTTTTCAATTTGGCCGTGGCTGATTCTCCTTATGATCTGCTGCTGCTTTCGGACAGAC
 TATTACCTCAGACGTAGACACTGGGCTTTTGGGGACATTCCCTGCCGAGTGGGGCTCTTCACGTTGGCCATGA
 ACAGGGCCGGGAGCATCGTGTCTTACGGTGGTGGCTGCGGACAGGTATTTCAAAGTGGTCCACCCCCACCA
 CGCGGTGAACACTATCTCCACCCGGGTGGCGGCTGGCATCGTCTGCACCCTGTGGGCCCTGGTCATCCTGGGA
 ACAGTGTATCTTTTGGTGGAGAACCATCTCTGCGTGCAAGAGACGGCCGTCTCCTGTGAGAGCTTCATCATGG
 AGTCGGCCAATGGCTGGCATGACATCATGTTCCAGCTGGAGTTCTTTATGCCCTCGGCATCATCTTATTTTG
 CTCTTCAAGATTGTTTGGAGCCTGAGGCGGAGGCAGCAGCTGGCCAGACAGGCTCGGATGAAGAAGGCGACC
 CGGTTTCATCATGTTGGTGGCAATTGTGTTTCATCACATGCTACCTGCCAGCGTGTCTGCTAGACTCTATTTC
 TCTGGACGGTGCCTCGAGTGCCTGCGATCCCTCTGTCCATGGGGCCCTGCACATAACCCTCAGCTTCACCTA
 CATGAACAGCATGCTGGATCCCCTGGTGTATTATTTTCAAGCCCTCCTTTCCAAATTCTACAACAGCTC
 AAAATCTGCAGTCTGAAACCAAGCAGCCAGGACACTCAAAACACAAAGGCCGGAAGAGATGCCAATTTCTGA
 ACCTCGGTTCGCGAGGAGTTGCATCAGTGTGGCAAATAGTTTCCAAAGCCAGTCTGATGGGCAATGGGATCCCCA
 CATTGTTGAGTGGCACTGA

The following amino acid sequence <SEQ ID NO. 80> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 79:

MYNGSCCRIEGDTISQVMPPLILIVAFVLGALNGVALCGFCFHMKTWKPSTVYLENLAVADFLLMICLPFRTD
YYLRRRHWAFGDIPCRVGLFTLAMNRAGSIVFLTVVAADRYFKVVPHHAVNTISTRVAAGIVCTLWALVILG
TVYLLLENHLVCQETAVSCSFIMESANGWHDIMFQLEFFMPLGIILFCFKIVWSLRRRQQLARQARMKKAT
RFIMVVAIVFITCYLPSVSARLYFLWTVPSACDPSVHGALHITLSFTYMNSMLDPLVYYFSSPSFPKFNKL
KICSLKPKQPGHSKTQRPEMPFISNLGRRSCISVANSFQSQSDGQWDPHIVEWH

The following DNA sequence nGPCR-16 <SEQ ID NO. 81> was identified in *H. sapiens*:

ATGACAGGTGACTTCCCAAGTATGCCTGGCCACAATACCTCCAGGAATTCCTCTTGCATCCTATAGACACCC
 CACTTAATCAGCCTCTACTTCATAGTGCTTATTGGCGGGCTGGTGGGTGTCAATTCCATTCTTTCTCTCTGG
 TGAAAATGAACACCCGGTCAGTGACCACCATGGCGGTCACTTAACCTGGTGGTGGTCCACAGCGCTTTTCTGCT
 GACAGTGCCATTCGCTTGACCTACCTCATCAAGAAGACTTGGATGTTTGGGCTGCCCTTCTGCAAAATTTGTG

AGTGCCATGCTGCACATCCACATGTACCTCACGTTCTTCTATGTGGTGATCCTGGTCACCAGATACCTCA
TCTTCTTCAAGTGCAAGACAAAGTGGAAATCTACAGAAAAGTGCATGCTGTGGCTGCCAGTCTGGCATGTG
GACGCTGGTGATTGTCAATTGTGGTACCCCTGGTGTCTCCCGGTATGGAATCCATGAGGAATACAATGAGGAG
CACTGTTTTAAATTCACAAAGAGCTTGGCTTACACATATGTGAAAATCATCAACTATATGATAGTCATTTTG
TCATAGCCGTTGCTGTGATTCTGTTGGTCTTCCAGGTCTTCATCATTATGTTGATGGTGCAGAAGCTACGCCA
CTCTTTACTATCCCACCAGGAGTCTGGGCTCAGCTGAAAACCTATTTTTATAGGGGTCATCCTTGTGTTGT
TTCCTTCCCTACCAGTCTTTTAGGATCTATTACTTGAATGTTGTGACGCATTCCAATGCCTGTAACAGCAAGG
TTGCATTTTATAACGAAATCTTCTGAGTGTAAACAGCAATTAGCTGCTATGATTGCTTCTCTTGTCTTTGG
GGGAAGCCATTGGTTTAAAGCAAAAGATAATTGGCTTATGGAATTGTGTTTGTGCGGTAGCCACAACTACA
GTATTCATATTTGCTTCCTTTATATTGGGAATAAAAATGGGTATAGGGGAGGTAAAGATGGTATTTTCATTACT
TGATCAAAACCATGCCTTGATGTACCCAAAACAAAAGGACTATAAAATGCAAGAGCCCTCATTGTAGTCCTTA
TGGGATCCCTCCCATCTCTGAGTGATGGCCGTACAAAGACCAGTGTGTTGAATCCACCTGGAGTTGCAATAT
TACATTATTTCCAGTACAGAAATGTCTGTGTGGCCCATGAAAGCAACATAGGTTTAAAGAGTTTATAGAGTTTC
ATTAGCTCATTCTAAGTTCCTCTGTTTGAAGCATGGTCTCTTAGGTTTGGACTGAACTCAGACCTTTAGTTC
TTTTCATCCCACTTACCTTAGGTAAGTAATTTCTGGCCACCACCAGCTCCAAGACACAACTCTCCTTCG
CTAACCAGGTTAGATGTCCCATTCATCTCATGCCCTGATAAAAACGATAAGGGGAGAGAATAGTTAAAAATT
TTTCTAGGGTATCATAACTCTGGTAGGAAGTCATCTGTCTAGAAATCAAGAGAAAAAGAACGTGTGGCCCTCT
GTTATAACAAGGGTTCTAGATTTGTCTGTGAAAGGTCGTTTAAAGACTTGGGGATCAACTTCCTCAATTAT
CACCAATTGCACGTGTGCTCCAAAATCATTAAAAAGCTTACTGGACATATCTACATAATGGTGAAACTGTAA
TTTAGAGACTACCTCTGACTAATGTGCTGGTAGGCATTAAGTTCCTCAAGGGAAGTGATTAAAAATTTT
TTCTCTCTGTTTTTGTAGAGAAATTTCTAGATGCTCTGGGCCACAGTTAATTAAGATTTTTTGGGGGGGACAGA
AAGTTATACTGAAATCTTTAGAGCTCCCTCCGCCGTAAAAATTATATATATATATATTTAAATTATACCTTA
AGTTCTGGGGTACATGTGCAGAAATGTGCAGGTTTGTACATAGGTATACACGTGCCATGGTGGTTTGGCGCAC
CTGTCAACCCATCTACATTAGGTATTTCTCCTAATGCTCTCCCTCCCTAGCCCCCACCCTGGACAGGCCC
CATTGTGTGATGTTCCCTCCCTGTGTCCATGTGTTTTCATTGTTCAACTCCCACTTCTAAGTGAGAACATGC
GGTGTTTGGTTTTCTGTTCCCTGTGTAGTTTGTCTGAGAAATGATGGTTTCCAGGTAAATTTATATATTTTAA
ATAAATGAAAACGTGTGTTTTTAAAGAGGACTTTTGAAGTATATAGAAAAACCATTAAATTTAGACTCTGTG
AGATTAGGTTGCATGAAGAAGTTTTCTGAATATTTGAAGAGTGGATAAATAAATGTCCCCAAAGCAATAAA
ATCATAATCCTTTAAATATAGGAAAAATACTAATGGGAAGTAGGCTTAATACTCGGGATGAAATAATCTGT
ACAACAACTCCCATGACACATGTTTACCTATGTAACAAACCTGCACATGTACCCTGAACCTAAATATAAAT
TTAAAGTATAATAATAAATAATATGGATTTCTTT

The following amino acid sequence <SEQ ID NO. 82> is the predicted amino acid sequence derived from the DNA sequence of SEQ ID NO. 81:

MTGDFPSPMPGHNTSRNSSCDPIVTPHLISLYFIVLIGGLVGVISILELLVKMNTSRVTTMAVINLVVHVSFEL
LTVPFRLTYLIKKTWMFGLPFCKFVSAMLIHMYLTFLEFYVVLVTRYLIFFKCKDKVEFYRKLHAVAASAGM
WTLVIVIVVPLVVSRYGIHEEYNEEHCFKFKELAYTYVKIINYMIVFVIAVAVILLVFQVFIIMLMVQKLR
HSLLSHQEFWAQLKNLFFIGVILVCFPLPYQFFRIYYLNVVTHSNACNSKVAFYNEIFLSVTAISCYDLLLFV
GGSHWFKQKIIGLWNCVLCR

The following DNA sequence nGPCR-40 <SEQ ID NO. 83> was identified in *H. sapiens*:

GCAGGAGCACTGAAAATCAGGAACAATCCTGATTTTTTGTGATAATCAACAAGGACAAAACCTCTCCATATG
TAAATAACAGCGTTATGAGCAGCAATTCATCCCTGCTGGTGGCTGTGCAGCTGTGCTACGCGAACGTGAATGG
GTCCTGTGTGAAAATCCCTTCTCGCCGGGATCCCGGGTGATTCTGTACATAGTGTGTTGGCTTTGGGGCTGTG
CTGGCTGTGTTTGGAAACCTCCTGGTGATGATTTCAATCCTCCATTCAAGCAGCTGCACCTCCGACCAATT
TTCTCGTTGCCTCTCTGGCCTGCGCTGATTTCTTGGTGGGTGTGACTGTGATGCCCTCAGCATGGTCAGGAC
GGTGGAGAGCTGCTGGTATTTTGGGAGGAGTTTTGTACTTTCCACACCTGCTGTGATGTGGCATTGTTGTAC
TCTTCTCTCTTCACTTGTGCTTCATCTCCATCGACAGGTACATTGCGGTTACTGACCCCTGGTCTATCCTA
CCAAGTTACACGTATCTGTGTCAGGAATTTGCATCAGCGTGTCTGGATCCTGCCCTCATGTACAGCGGTGC
TGTGTTCTACACAGGTGCTATGACGATGGGCTGGAGGAATTATCTGATGCCCTAAACTGTATAGGAGTTGT
CAGACCGTTGTAAATCAAACTGGGTGTTGACAGATTTCTATCCTTCTTTATACCTACCTTTATTATGATAA
TTCTGTATGGTAACATATTTCTTGTGGCTAGACGACAGGCGAAAAAGATAGAAAATACTGGTAGCAAGACAGA
ATCATCCTCAGAGAGTTACAAAGCCAGAGTGGCCAGGAGAGAGAGAAAAGCAGCTAAAACCTGGGGGTACA
GTGGTAGCATTATGATTTTCATGGTTACCATATAGCATTGATTCTTAATTGATGCCTTTATGGGCTTTATAA
CCCTGCGCTGATTTATGAGATTTGCTGTTGGTGTGCTTATTATAACTCAGCCATGAATCCTTTGATTTATGC
TTTATTTTACCCATGGTTTAGGAAAGCAATAAAGTTATTGTAAGTGGTCAGGTTTTAAAGAACAGTTACGA
ACCATGAATTTGTTTCTGAACATATATA

for 30 sec, 55°C for 30 sec, 68°C for 2 min. The PCR reaction was loaded onto a 1.2 % agarose gel. The DNA band was excised from the gel, placed in GenElute Agarose spin column (Supelco) and spun for 10 min at maximum speed in a microcentrifuge. The eluted DNA was EtOH precipitated and resuspended in 4µl H₂O for ligation. The forward PCR primer sequence was:

LW1564: GCATAAGCTTCCATGTACAACGGGTCGTGCTGC (SEQ ID NO: 107), and the reverse PCR primer was:

LW1565: GCATTCTAGATCAGTGCCACTCAACAATGTGGG (SEQ ID NO: 108).

The ligation reaction used solutions from the TOPO TA Cloning Kit (Invitrogen) which consisted of 4µl PCR product DNA and 1 µl pCRII-TOPO vector that was incubated for 5 minutes at room temperature. To the ligation reaction one microliter of 6X TOPO Cloning Stop Solution was added then the reaction was placed on ice. Two microliters of the ligation reaction was transformed in One Shot TOP10 cells (Invitrogen), and placed on ice for 30 minutes. The cells were heat-shocked for 30 seconds at 42°C, placed on ice for two minutes, 250 µl of SOC was added, then incubated at 37°C with shaking for one hour and then plated onto ampicillin plates. A single colony containing an insert was used to inoculate a 5 ml culture of LB medium. Plasmid DNA was purified using a Concert Rapid Plasmid Miniprep System (GibcoBRL) and then sequenced.

The DNA subcloned into pCRII was sequenced using the ABI PRISMTM 310 Genetic Analyzer (PE Applied Biosystems) which uses advanced capillary electrophoresis technology and the ABI PRISMTM BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit. Each cycle-sequencing reaction contained 6 µl of H₂O, 8 µl of BigDye Terminator mix, 5 µl mini-prep DNA (0.1 µg/µl), and 1 µl primer (25 ng/µl) and was performed in a Perkin-Elmer 9600 thermocycler with 25 cycles of 96°C for 10 sec, 50°C for 10 sec, and 60°C for 4 min. The product was purified using a CentriflexTM gel filtration cartridge, dried under vacuum, then dissolved in 16 µl of Template Suppression Reagent (PE Applied Biosystems). The samples were heated at 95°C for 5 min then placed in the 310 Genetic Analyzer, yielding the sequence of SEQ ID NO: 79.

30 nGPCR-16: PCR AND SUBCLONING

PCR was performed in a 50 µl reaction containing 32 µl H₂O, 5 µl 10X TT buffer (140 mM Ammonium Sulfate, 0.1 % gelatin, 0.6 M Tris-tricine pH 8.4), 5 µl 15 mM MgSO₄, 2 µl 10 mM dNTP, 5 µl 2445704H1 DNA (0.17 Tg/Tl), 0.3 µl of LW1587 (1 µg/µl), 0.3 µl

of LW1588 (1 $\mu\text{g}/\mu\text{l}$), 0.4 μl High Fidelity Taq polymerase (Boehringer Mannheim). The PCR reaction was performed on a Robocycler thermocycler (Stratagene) starting with 1 cycle of 94°C for 2 min followed by 15 cycles of 94°C for 30 sec, 55°C for 1.3 min, 68°C for 2 min. The PCR reaction was loaded onto a 1.2 % agarose gel. The DNA band was excised
5 from the gel, placed in GenElute Agarose spin column (Supelco) and spun for 10 min at maximum speed in a microcentrifuge. The eluted DNA was EtOH precipitated and resuspended in 12 μl H₂O for ligation. The PCR primer sequence for the forward primer was:

LW1587: GATCAAGCTTATGACAGGTGACTTCCCAAGTATGC (SEQ ID NO: 111), and the sequence for the reverse primer was:

10 LW1588: GATCCTCGAGGCTAACGGCACAAAACACAATTCC (SEQ ID NO: 112).

The ligation reaction used solutions from the TOPO TA Cloning Kit (Invitrogen) which consisted of 4 μl PCR product DNA and 1 μl pCRII-TOPO vector that was incubated for 5 minutes at room temperature. To the ligation reaction one microliter of 6X TOPO
15 Cloning Stop Solution was added then the reaction was placed on ice. Two microliters of the ligation reaction was transformed in One-Shot TOP10 cells (Invitrogen), and placed on ice for 30 minutes. The cells were heat-shocked for 30 seconds at 42°C, placed on ice for two minutes, 250 μl of SOC was added, then incubated at 37°C with shaking for one hour and then plated onto ampicillin plates. A single colony containing an insert was used to inoculate
20 a 5 ml culture of LB medium. Plasmid DNA was purified using a Concert Rapid Plasmid Miniprep System (GibcoBRL) and then sequenced.

The DNA subcloned into pCRII was sequenced using the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems) which uses advanced capillary electrophoresis technology and the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit. Each
25 cycle-sequencing reaction contained 6 μl of H₂O, 8 μl of BigDye Terminator mix, 5 μl mini-prep DNA (0.1 $\mu\text{g}/\mu\text{l}$), and 1 μl primer (25 ng/ μl) and was performed in a Perkin-Elmer 9600 thermocycler with 25 cycles of 96°C for 10 sec, 50°C for 10 sec, and 60°C for 4 min. The product was purified using a Centriflex™ gel filtration cartridge, dried under vacuum, then dissolved in 16 μl of Template Suppression Reagent (PE Applied Biosystems). The samples
30 were heated at 95°C for 5 min then placed in the 310 Genetic Analyzer, yielding the sequence of SEQ ID NO: 81.

EXAMPLE 4: TISSUE EXPRESSION PROFILING

Tissue specific expression of the cDNAs encoding nGPCR-1, nGPCR-3, nGPCR-9, nGPCR-11, nGPCR-16, nGPCR-40, nGPCR-54, nGPCR-56, and nGPCR-58 was detected using a PCR-based system. Tissue specific expression of cDNAs encoding nGPCR-x may be accomplished using similar methods.

Primers were synthesized by Genosys Corp., The Woodlands, TX. PCR reactions were assembled using the components of the Expand Hi-Fi PCR System™ (Roche Molecular Biochemicals, Indianapolis, IN).

nGPCR-1

The RapidScan™ Gene Expression Panel was used to generate a comprehensive expression profile of the putative GPCR in human tissues. Human tissues in the array may include: brain, heart, kidney, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary gland, thyroid, adrenal gland, pancreas, ovary, uterus, prostate, skin, PBL, bone marrow, fetal brain, fetal liver. Human brain regions in the array may include: frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla and spinal cord.

Expression of the nGPCR-1 in the various tissues was detected by using PCR primers designed based on the available sequence of the receptor that will prime the synthesis of a 212bp fragment in the presence of the appropriate cDNA. The forward primer was:

GCTCAACCCACTCATCTATGCC (SEQ ID NO: 97), and the reverse primer was: AAAGTTCTCTGCCCTTACCGTC (SEQ ID NO: 98)

The PCR reaction mixture was added to each well of the PCR plate. The plate was placed in a GeneAmp PCR9700 PCR thermocycler (Perkin Elmer Applied Biosystems). The plate was then exposed to the following cycling parameters: Pre-soak 94°C for 3 min; denaturation at 94°C for 30 seconds; annealing at primer T_m for 45 seconds; extension 72°C for 2 minutes; for 35 cycles. PCR products were then separated and analyzed by electrophoresis on a 1.5-% agarose gel.

The 4-log dilution range of cDNA deposited on the plate ensured that the amplification reaction is within the linear range and, hence, facilitated the semi-quantitative determination of relative mRNA accumulation in the various tissues or brain regions examined.

Expression of nGPCR-1 was found to be highest in the testis, adrenal gland and heart. Significant levels of expression were also found in the brain, kidney, spleen ovary, prostate, muscle, PBL, stomach and bone marrow. Within the brain, expression levels were highest in

determination of relative mRNA accumulation in the various tissues or brain regions examined.

nGPCR-11 was expressed in the thyroid gland, brain, heart, kidney, adrenal gland, spleen, liver, ovary, muscle, testis, salivary gland, colon, prostate, small intestine, skin
5 stomach, bone marrow, fetal brain and placenta. Within the brain, nGPCR-11 was expressed in the temporal lobe, amygdala, substantia nigra, pons, spinal cord, frontal lobe, and cerebellum.

Expression of the nGPCR-11 in the brain provided an indication that modulators of nGPCR-11 activity have utility for treating disorders, including but not limited to,
10 schizophrenia, affective disorders, metabolic disorders, inflammatory disorders, cancers, ADHD/ADD (*i.e.*, Attention Deficit-Hyperactivity Disorder/Attention Deficit Disorder), and neural disorders such as Alzheimer's disease, Parkinson's disease, migraine, and senile dementia. Some other diseases for which modulators of nGPCR-11 may have utility include depression, anxiety, bipolar disease, epilepsy, neuritis, neurasthenia, neuropathy, neuroses,
15 and the like. Use of nGPCR-11 modulators, including nGPCR-11 ligands and anti-nGPCR-11 antibodies, to treat individuals having such disease states is intended as an aspect of the invention.

Expression of nGPCR-11 in the thyroid gland, indicates that agonists or antagonists could be of use in the treatment of thyroid dysfunction such as thyreotoxicosis and
20 myxoedema. They could also be of use in the stimulation of thyroid hormone release leading to overall increase in metabolic rate and weight reduction. The expression of nGPCR-11 in liver and muscle indicate a use for agonists or antagonists in regulation of glucose metabolism applicable in diabetes type II.

nGCPR-16

25 The RapidScan™ Gene Expression Panel was used to generate a comprehensive expression profile of the putative GPCR in human tissues. Human tissues in the array included, *inter alia*: brain, heart, kidney, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary gland, thyroid, adrenal gland, pancreas, ovary, uterus, prostate, skin, PBL, bone marrow, fetal brain, fetal liver. Human brain regions in the array
30 included, *inter alia*: frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla and spinal cord.

Expression of nGPCR-16 in the various tissues was detected by using PCR primers designed based on the available sequence of the receptor that will prime the synthesis of a

205bp fragment in the presence of the appropriate cDNA. The forward primer used to detect expression of nGPCR-16 was:

5' CAGCCCAAACATCCAAGTC 3'. (SEQ ID NO: 113). The reverse primer used to detect expression of nGPCR-16 was:

5' ACCCCACTTAATCAGCCTC 3' (SEQ ID NO: 114).

For detection of expression within brain regions, the same primer set was used with the Human Brain Rapid Scan™ Panel (OriGene Technologies, Rockville, MD). This panel represents serial dilutions over a 2 log range of first strand cDNA from the following brain regions arrayed in a 96 well format: frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla and spinal cord.

Twenty-five microliters of the PCR reaction mixture was added to each well of the RapidScan PCR plate. The plate was placed in a GeneAmp 9700 PCR thermocycler (Perkin Elmer Applied Biosystems). The following cycling program was executed: Pre-soak at (94° for 3min.) followed by 35 cycles of [(94°C for 45 sec.) (53°C for 2 min.) (72°C for 45 sec.)]. PCR reaction products were then separated and analyzed by electrophoresis on a 2.0% agarose gel, and stained with ethidium bromide.

The 4-log dilution range of cDNA deposited on the plate ensured that the amplification reaction was within the linear range and, facilitated semi-quantitative determination of relative mRNA accumulation in the various tissues or brain regions examined.

nGPCR-16 was expressed in the ovary, lung, prostate, bone marrow, salivary gland, heart, adrenal gland, spleen, liver, small intestine, skin, muscle, peripheral blood leukocytes, testis, placenta, fetal liver, brain, thyroid gland, kidney, pancreas, colon, uterus, and stomach.. Within the brain, nGPCR-16 was expressed in all areas examined including the frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla and spinal cord.

Expression of nGPCR-16 in the brain provides an indication that modulators of nGPCR-16 activity have utility for treating neurological disorders, including but not limited to, schizophrenia, affective disorders, ADHD/ADD (*i.e.*, Attention Deficit-Hyperactivity Disorder/Attention Deficit Disorder), and neural disorders such as Alzheimer's disease, Parkinson's disease, migraine, and senile dementia. Some other diseases for which modulators of nGPCR-16 may have utility include depression, anxiety, bipolar disease, epilepsy, neuritis, neurasthenia, neuropathy, neuroses, and the like. Use of nGPCR-16

modulators, including nGPCR-16 ligands and anti-nGPCR-16 antibodies, to treat individuals having such disease states is intended as an aspect of the invention.

nGPCR-40

The RapidScan™ Gene Expression Panel (OriGene Technologies, Rockville, MD) was used to generate a comprehensive expression profile of the putative GPCR in human tissues. Human tissues arrayed include: brain, heart, kidney, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary gland, thyroid, adrenal gland, pancreas, ovary, uterus, prostate, skin, PBL, bone marrow, fetal brain, fetal liver. The forward primer used was:

5'ACAGCCCCAAAGCCAAACAC3', (SEQ ID NO: 117), and the reverse primer was:

5'CCGCAGGAGCAATGAAAATCAG3', (SEQ ID NO: 118). This primer set primed the synthesis of a 220 base pair fragment in the presence of the appropriate cDNA. For detection of expression within brain regions, the same primer set was used with the Human Brain RapidScan™ Panel (OriGene Technologies, Rockville, MD). This panel represents serial dilutions over a 2 log range of first strand cDNA from the following brain regions arrayed in a 96 well format: frontal lobe, temporal lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla and spinal cord.

Twenty-five microliters of the PCR reaction mixture was added to each well of the RapidScan PCR plate. The plate was placed in a GeneAmp 9700 PCR thermocycler (Perkin Elmer Applied Biosystems). The following cycling program was executed: Pre-soak at (94°C for 3min.) followed by 35 cycles of [(94° for 45 sec.) (54°C for 2 min.) (72° for 45 sec.)]. PCR reaction products were then separated and analyzed by electrophoresis on a 2.0% agarose gel stained with ethidium bromide.

The dilution range of cDNA deposited on the plates ensured that the amplification reaction was within the linear range and, hence, facilitated semi-quantitative determination of relative mRNA accumulation in the various tissues or brain regions examined.

nGPCR-40 was expressed in the brain, peripheral blood lymphocytes, pancreas, ovary, uterus, testis, salivary gland, kidney, adrenal gland, liver, bone marrow, prostate, fetal liver, colon, muscle, and fetal brain, may be found in many other tissues, including, but not limited to, lung, small intestine, fetal brain cord, and bone. Within the brain, nGPCR-40 was expressed in the frontal lobe, hypothalamus, pons, cerebellum, caudate nucleus, and medulla.

EXAMPLE 10: ASSAYS TO IDENTIFY MODULATORS OF nGPCR-X ACTIVITY

Set forth below are several nonlimiting assays for identifying modulators (agonists and antagonists) of nGPCR-x activity. Among the modulators that can be identified by these assays are natural ligand compounds of the receptor; synthetic analogs and derivatives of natural ligands; antibodies, antibody fragments, and/or antibody-like compounds derived from natural antibodies or from antibody-like combinatorial libraries; and/or synthetic compounds identified by high-throughput screening of libraries; and the like. All modulators that bind nGPCR-x are useful for identifying nGPCR-x in tissue samples (*e.g.*, for diagnostic purposes, pathological purposes, and the like). Agonist and antagonist modulators are useful for up-regulating and down-regulating nGPCR-x activity, respectively, to treat disease states characterized by abnormal levels of nGPCR-x activity. The assays may be performed using single putative modulators, and/or may be performed using a known agonist in combination with candidate antagonists (or *visa versa*).

A. cAMP Assays

In one type of assay, levels of cyclic adenosine monophosphate (cAMP) are measured in nGPCR-x-transfected cells that have been exposed to candidate modulator compounds. Protocols for cAMP assays have been described in the literature. (See, *e.g.*, Sutherland *et al.*, *Circulation* 37: 279 (1968); Frandsen *et al.*, *Life Sciences* 18: 529-541 (1976); Dooley *et al.*, *Journal of Pharmacology and Experimental Therapeutics* 283 (2): 735-41 (1997); and George *et al.*, *Journal of Biomolecular Screening* 2 (4): 235-40 (1997)). An exemplary protocol for such an assay, using an Adenylyl Cyclase Activation FlashPlate® Assay from NEN™ Life Science Products, is set forth below.

Briefly, the nGPCR-x coding sequence (*e.g.*, a cDNA or intronless genomic DNA) is subcloned into a commercial expression vector, such as pzeoSV2 (Invitrogen), and transiently transfected into Chinese Hamster Ovary (CHO) cells using known methods, such as the transfection protocol provided by Boehringer-Mannheim when supplying the FuGENE 6 transfection reagent. Transfected CHO cells are seeded into 96-well microplates from the FlashPlate® assay kit, which are coated with solid scintillant to which antisera to cAMP has been bound. For a control, some wells are seeded with wild type (untransfected) CHO cells. Other wells in the plate receive various amounts of a cAMP standard solution for use in creating a standard curve.

One or more test compounds (*i.e.*, candidate modulators) are added to the cells in each well, with water and/or compound-free medium/diluent serving as a control or controls.

After treatment, cAMP is allowed to accumulate in the cells for exactly 15 minutes at room temperature. The assay is terminated by the addition of lysis buffer containing [125 I]-labeled cAMP, and the plate is counted using a Packard TopcountTM 96-well microplate scintillation counter. Unlabeled cAMP from the lysed cells (or from standards) and fixed amounts of [125 I]-cAMP compete for antibody bound to the plate. A standard curve is constructed, and cAMP values for the unknowns are obtained by interpolation. Changes in intracellular cAMP levels of cells in response to exposure to a test compound are indicative of nGPCR-x modulating activity. Modulators that act as agonists of receptors which couple to the G_s subtype of G proteins will stimulate production of cAMP, leading to a measurable 3-10 fold increase in cAMP levels. Agonists of receptors which couple to the G_{i/o} subtype of G proteins will inhibit forskolin-stimulated cAMP production, leading to a measurable decrease in cAMP levels of 50-100%. Modulators that act as inverse agonists will reverse these effects at receptors that are either constitutively active or activated by known agonists.

B. Aequorin Assays

In another assay, cells (e.g., CHO cells) are transiently co-transfected with both a nGPCR-x expression construct and a construct that encodes the photoprotein apoaequorin. In the presence of the cofactor coelenterazine, apoaequorin will emit a measurable luminescence that is proportional to the amount of intracellular (cytoplasmic) free calcium. (See generally, Cobbold, *et al.* "Aequorin measurements of cytoplasmic free calcium," *In* McCormack J.G. and Cobbold P.H., eds., *Cellular Calcium: A Practical Approach*. Oxford: IRL Press (1991); Stables *et al.*, *Analytical Biochemistry* 252: 115-26 (1997); and Haugland, *Handbook of Fluorescent Probes and Research Chemicals*. Sixth edition. Eugene OR: Molecular Probes (1996).)

In one exemplary assay, nGPCR-x is subcloned into the commercial expression vector pzeoSV2 (Invitrogen) and transiently co-transfected along with a construct that encodes the photoprotein apoaequorin (Molecular Probes, Eugene, OR) into CHO cells using the transfection reagent FuGENE 6 (Boehringer-Mannheim) and the transfection protocol provided in the product insert.

The cells are cultured for 24 hours at 37°C in MEM (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin and 10 µg/ml streptomycin, at which time the medium is changed to serum-free MEM containing 5 µM coelenterazine (Molecular Probes, Eugene, OR). Culturing is then continued for two

additional hours at 37°C. Subsequently, cells are detached from the plate using VERSEN (Gibco/BRL), washed, and resuspended at 200,000 cells/ml in serum-free MEM.

Dilutions of candidate nGPCR-x modulator compounds are prepared in serum-free MEM and dispensed into wells of an opaque 96-well assay plate at 50 µl/well. Plates are then loaded onto an MLX microtiter plate luminometer (Dynex Technologies, Inc., Chantilly, VA). The instrument is programmed to dispense 50 µl cell suspensions into each well, one well at a time, and immediately read luminescence for 15 seconds. Dose-response curves for the candidate modulators are constructed using the area under the curve for each light signal peak. Data are analyzed with SlideWrite, using the equation for a one-site ligand, and EC₅₀ values are obtained. Changes in luminescence caused by the compounds are considered indicative of modulatory activity. Modulators that act as agonists at receptors which couple to the G_q subtype of G proteins give an increase in luminescence of up to 100 fold. Modulators that act as inverse agonists will reverse this effect at receptors that are either constitutively active or activated by known agonists.

C. Luciferase Reporter Gene Assay

The photoprotein luciferase provides another useful tool for assaying for modulators of nGPCR-x activity. Cells (*e.g.*, CHO cells or COS 7 cells) are transiently co-transfected with both a nGPCR-x expression construct (*e.g.*, nGPCR-x in pzeoSV2) and a reporter construct which includes a gene for the luciferase protein downstream from a transcription factor binding site, such as the cAMP-response element (CRE), AP-1, or NF-kappa B. Agonist binding to receptors coupled to the G_s subtype of G proteins leads to increases in cAMP, thereby activating the CRE transcription factor and resulting in expression of the luciferase gene. Agonist binding to receptors coupled to the G_q subtype of G protein leads to production of diacylglycerol that activates protein kinase C, which activates the AP-1 or NF-kappa B transcription factors, in turn resulting in expression of the luciferase gene. Expression levels of luciferase reflect the activation status of the signaling events. (See generally, George *et al.*, Journal of Biomolecular Screening 2(4): 235-240 (1997); and Stratowa *et al.*, Current Opinion in Biotechnology 6: 574-581 (1995)). Luciferase activity may be quantitatively measured using, *e.g.*, luciferase assay reagents that are commercially available from Promega (Madison, WI).

In one exemplary assay, CHO cells are plated in 24-well culture dishes at a density of 100,000 cells/well one day prior to transfection and cultured at 37°C in MEM (Gibco/BRL) supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin and 10

µg/ml streptomycin. Cells are transiently co-transfected with both a nGPCR-x expression construct and a reporter construct containing the luciferase gene. The reporter plasmids CRE-luciferase, AP-1-luciferase and NF-kappaB-luciferase may be purchased from Stratagene (LaJolla, CA). Transfections are performed using the FuGENE 6 transfection reagent (Boehringer-Mannheim) according to the supplier's instructions. Cells transfected with the reporter construct alone are used as a control. Twenty-four hours after transfection, cells are washed once with PBS pre-warmed to 37°C. Serum-free MEM is then added to the cells either alone (control) or with one or more candidate modulators and the cells are incubated at 37°C for five hours. Thereafter, cells are washed once with ice-cold PBS and lysed by the addition of 100 µl of lysis buffer per well from the luciferase assay kit supplied by Promega. After incubation for 15 minutes at room temperature, 15 µl of the lysate is mixed with 50 µl of substrate solution (Promega) in an opaque-white, 96-well plate, and the luminescence is read immediately on a Wallace model 1450 MicroBeta scintillation and luminescence counter (Wallace Instruments, Gaithersburg, MD).

Differences in luminescence in the presence versus the absence of a candidate modulator compound are indicative of modulatory activity. Receptors that are either constitutively active or activated by agonists typically give a 3 to 20-fold stimulation of luminescence compared to cells transfected with the reporter gene alone. Modulators that act as inverse agonists will reverse this effect.

D. Intracellular calcium measurement using FLIPR

Changes in intracellular calcium levels are another recognized indicator of G protein-coupled receptor activity, and such assays can be employed to screen for modulators of nGPCR-x activity. For example, CHO cells stably transfected with a nGPCR-x expression vector are plated at a density of 4×10^4 cells/well in Packard black-walled, 96-well plates specially designed to discriminate fluorescence signals emanating from the various wells on the plate. The cells are incubated for 60 minutes at 37°C in modified Dulbecco's PBS (D-PBS) containing 36 mg/L pyruvate and 1 g/L glucose with the addition of 1% fetal bovine serum and one of four calcium indicator dyes (Fluo-3™ AM, Fluo-4™ AM, Calcium Green™-1 AM, or Oregon Green™ 488 BAPTA-1 AM), each at a concentration of 4 µM. Plates are washed once with modified D-PBS without 1% fetal bovine serum and incubated for 10 minutes at 37°C to remove residual dye from the cellular membrane. In addition, a series of washes with modified D-PBS without 1% fetal bovine serum is performed immediately prior to activation of the calcium response.

A calcium response is initiated by the addition of one or more candidate receptor agonist compounds, calcium ionophore A23187 (10 μ M; positive control), or ATP (4 μ M; positive control). Fluorescence is measured by Molecular Device's FLIPR with an argon laser (excitation at 488 nm). (See, *e.g.*, Kuntzweiler *et al.*, Drug Development Research, 44(1):14-20 (1998)). The F-stop for the detector camera was set at 2.5 and the length of exposure was 0.4 milliseconds. Basal fluorescence of cells was measured for 20 seconds prior to addition of candidate agonist, ATP, or A23187, and the basal fluorescence level was subtracted from the response signal. The calcium signal is measured for approximately 200 seconds, taking readings every two seconds. Calcium ionophore A23187 and ATP increase the calcium signal 200% above baseline levels. In general, activated GPCRs increase the calcium signal approximately 10-15% above baseline signal.

E. Mitogenesis Assay

In a mitogenesis assay, the ability of candidate modulators to induce or inhibit nGPCR-x-mediated cell division is determined. (See, *e.g.*, Lajiness *et al.*, Journal of Pharmacology and Experimental Therapeutics 267(3): 1573-1581 (1993)). For example, CHO cells stably expressing nGPCR-x are seeded into 96-well plates at a density of 5000 cells/well and grown at 37°C in MEM with 10% fetal calf serum for 48 hours, at which time the cells are rinsed twice with serum-free MEM. After rinsing, 80 μ l of fresh MEM, or MEM containing a known mitogen, is added along with 20 μ l MEM containing varying concentrations of one or more candidate modulators or test compounds diluted in serum-free medium. As controls, some wells on each plate receive serum-free medium alone, and some receive medium containing 10% fetal bovine serum. Untransfected cells or cells transfected with vector alone also may serve as controls.

After culture for 16-18 hours, 1 μ Ci of [3 H]-thymidine (2 Ci/mmol) is added to the wells and cells are incubated for an additional 2 hours at 37°C. The cells are trypsinized and collected on filter mats with a cell harvester (Tomtec); the filters are then counted in a Betaplate counter. The incorporation of [3 H]-thymidine in serum-free test wells is compared to the results achieved in cells stimulated with serum (positive control). Use of multiple concentrations of test compounds permits creation and analysis of dose-response curves using the non-linear, least squares fit equation: $A = B \times [C / (D + C)] + G$ where A is the percent of serum stimulation; B is the maximal effect minus baseline; C is the EC_{50} ; D is the concentration of the compound; and G is the maximal effect. Parameters B, C and G are determined by Simplex optimization.

Agonists that bind to the receptor are expected to increase [^3H]-thymidine incorporation into cells, showing up to 80% of the response to serum. Antagonists that bind to the receptor will inhibit the stimulation seen with a known agonist by up to 100%.

F. [^{35}S]-GTP γS Binding Assay

5 Because G protein-coupled receptors signal through intracellular G proteins whose activity involves GTP binding and hydrolysis to yield bound GDP, measurement of binding of the non-hydrolyzable GTP analog [^{35}S]-GTP γS in the presence and absence of candidate modulators provides another assay for modulator activity. (See, e.g., Kowal *et al.*, *Neuropharmacology* 37:179-187 (1998).)

10 In one exemplary assay, cells stably transfected with a nGPCR-x expression vector are grown in 10 cm tissue culture dishes to subconfluence, rinsed once with 5 ml of icecold $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free phosphate-buffered saline, and scraped into 5 ml of the same buffer. Cells are pelleted by centrifugation (500 x g, 5 minutes), resuspended in TEE buffer (25 mM Tris, pH 7.5, 5 mM EDTA, 5 mM EGTA), and frozen in liquid nitrogen. After thawing, the cells
15 are homogenized using a Dounce homogenizer (one ml TEE per plate of cells), and centrifuged at 1,000 x g for 5 minutes to remove nuclei and unbroken cells.

The homogenate supernatant is centrifuged at 20,000 x g for 20 minutes to isolate the membrane fraction, and the membrane pellet is washed once with TEE and resuspended in binding buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl_2 , 1 mM EDTA). The
20 resuspended membranes can be frozen in liquid nitrogen and stored at -70°C until use.

Aliquots of cell membranes prepared as described above and stored at -70°C are thawed, homogenized, and diluted into buffer containing 20 mM HEPES, 10 mM MgCl_2 , 1 mM EDTA, 120 mM NaCl, 10 μM GDP, and 0.2 mM ascorbate, at a concentration of 10-50 $\mu\text{g}/\text{ml}$. In a final volume of 90 μl , homogenates are incubated with varying concentrations of
25 candidate modulator compounds or 100 μM GTP for 30 minutes at 30°C and then placed on ice. To each sample, 10 μl guanosine 5'-O-(3[^{35}S]-thio) triphosphate (NEN, 1200 Ci/mmol; [^{35}S]-GTP γS), was added to a final concentration of 100-200 pM. Samples are incubated at 30°C for an additional 30 minutes, 1 ml of 10 mM HEPES, pH 7.4, 10 mM MgCl_2 , at 4°C is added and the reaction is stopped by filtration.

30 Samples are filtered over Whatman GF/B filters and the filters are washed with 20 ml ice-cold 10 mM HEPES, pH 7.4, 10 mM MgCl_2 . Filters are counted by liquid scintillation spectroscopy. Nonspecific binding of [^{35}S]-GTP γS is measured in the presence of 100 μM GTP and subtracted from the total. Compounds are selected that modulate the amount of

[³⁵S]-GTPγS binding in the cells, compared to untransfected control cells. Activation of receptors by agonists gives up to a five-fold increase in [³⁵S]GTPγS binding. This response is blocked by antagonists.

G. MAP Kinase Activity Assay

5 Evaluation of MAP kinase activity in cells expressing a GPCR provides another assay to identify modulators of GPCR activity. (See, *e.g.*, Lajiness *et al.*, Journal of Pharmacology and Experimental Therapeutics 267(3):1573-1581 (1993) and Boulton *et al.*, Cell 65:663-675 (1991).)

10 In one embodiment, CHO cells stably transfected with nGPCR-x are seeded into 6-well plates at a density of 70,000 cells/well 48 hours prior to the assay. During this 48-hour period, the cells are cultured at 37°C in MEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin and 10 µg/ml streptomycin. The cells are serum-starved for 1-2 hours prior to the addition of stimulants.

15 For the assay, the cells are treated with medium alone or medium containing either a candidate agonist or 200 nM Phorbol ester- myristoyl acetate (*i.e.*, PMA, a positive control), and the cells are incubated at 37°C for varying times. To stop the reaction, the plates are placed on ice, the medium is aspirated, and the cells are rinsed with 1 ml of icecold PBS containing 1 mM EDTA. Thereafter, 200 µl of cell lysis buffer (12.5 mM MOPS, pH 7.3, 12.5 mM glycerophosphate, 7.5 mM MgCl₂, 0.5 mM EGTA, 0.5 mM sodium vanadate, 1
20 mM benzamidine, 1 mM dithiothreitol, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 2 µg/ml pepstatin A, and 1 µM okadaic acid) is added to the cells. The cells are scraped from the plates and homogenized by 10 passages through a 23 3/4 G needle, and the cytosol fraction is prepared by centrifugation at 20,000 x g for 15 minutes.

25 Aliquots (5-10 µl containing 1-5 µg protein) of cytosol are mixed with 1 mM MAPK Substrate Peptide (APRTPGGRR (SEQ ID NO: 129), Upstate Biotechnology, Inc., N.Y.) and 50 µM [γ-³²P]ATP (NEN, 3000 Ci/mmol), diluted to a final specific activity of ~2000 cpm/pmol, in a total volume of 25 µl. The samples are incubated for 5 minutes at 30°C, and reactions are stopped by spotting 20 µl on 2 cm² squares of Whatman P81 phosphocellulose paper. The filter squares are washed in 4 changes of 1% H₃PO₄, and the squares are
30 subjected to liquid scintillation spectroscopy to quantitate bound label. Equivalent cytosolic extracts are incubated without MAPK substrate peptide, and the bound label from these samples are subtracted from the matched samples with the substrate peptide. The cytosolic extract from each well is used as a separate point. Protein concentrations are determined by a

dye binding protein assay (Bio-Rad Laboratories). Agonist activation of the receptor is expected to result in up to a five-fold increase in MAPK enzyme activity. This increase is blocked by antagonists.

H. [³H]Arachidonic Acid Release

5 The activation of GPCRs also has been observed to potentiate arachidonic acid release in cells, providing yet another useful assay for modulators of GPCR activity. (*See, e.g., Kanterman et al., Molecular Pharmacology 39:364-369 (1991).*) For example, CHO cells that are stably transfected with a nGPCR-x expression vector are plated in 24-well plates at a density of 15,000 cells/well and grown in MEM medium supplemented with 10% fetal
10 bovine serum, 2 mM glutamine, 10 U/ml penicillin and 10 µg/ml streptomycin for 48 hours at 37°C before use. Cells of each well are labeled by incubation with [³H]-arachidonic acid (Amersham Corp., 210 Ci/mmol) at 0.5 µCi/ml in 1 ml MEM supplemented with 10 mM HEPES, pH 7.5, and 0.5% fatty-acid-free bovine serum albumin for 2 hours at 37°C. The cells are then washed twice with 1 ml of the same buffer.

15 Candidate modulator compounds are added in 1 ml of the same buffer, either alone or with 10 µM ATP and the cells are incubated at 37°C for 30 minutes. Buffer alone and mock-transfected cells are used as controls. Samples (0.5 ml) from each well are counted by liquid scintillation spectroscopy. Agonists which activate the receptor will lead to potentiation of the ATP-stimulated release of [³H]-arachidonic acid. This potentiation is blocked by
20 antagonists.

I. Extracellular Acidification Rate

In yet another assay, the effects of candidate modulators of nGPCR-x activity are assayed by monitoring extracellular changes in pH induced by the test compounds. (*See, e.g., Dunlop et al., Journal of Pharmacological and Toxicological Methods 40(1):47-55 (1998).*)

25 In one embodiment, CHO cells transfected with a nGPCR-x expression vector are seeded into 12 mm capsule cups (Molecular Devices Corp.) at 4 x 10⁵ cells/cup in MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10 U/ml penicillin, and 10 µg/ml streptomycin. The cells are incubated in this medium at 37°C in 5% CO₂ for 24 hours.

Extracellular acidification rates are measured using a Cytosensor microphysiometer
30 (Molecular Devices Corp.). The capsule cups are loaded into the sensor chambers of the microphysiometer and the chambers are perfused with running buffer (bicarbonate-free MEM supplemented with 4 mM L-glutamine, 10 units/ml penicillin, 10 µg/ml streptomycin, 26 mM NaCl) at a flow rate of 100 µl/minute. Candidate agonists or other agents are diluted into the

running buffer and perfused through a second fluid path. During each 60-second pump cycle, the pump is run for 38 seconds and is off for the remaining 22 seconds. The pH of the running buffer in the sensor chamber is recorded during the cycle from 43-58 seconds, and the pump is re-started at 60 seconds to start the next cycle. The rate of acidification of the running buffer during the recording time is calculated by the Cytosoft program. Changes in the rate of acidification are calculated by subtracting the baseline value (the average of 4 rate measurements immediately before addition of a modulator candidate) from the highest rate measurement obtained after addition of a modulator candidate. The selected instrument detects 61 mV/pH unit. Modulators that act as agonists of the receptor result in an increase in the rate of extracellular acidification compared to the rate in the absence of agonist. This response is blocked by modulators which act as antagonists of the receptor.

EXAMPLE 11: *IN SITU* HYBRIDIZATION

DNA Probe Preparation For nGPCR-11, -16, -40, -54, and -56

DNA probes for *in situ* hybridization were prepared as follows. Two sets of primer pairs were prepared. The first set has the sequence for T7 polymerase promoter on the 5' primer to make the sense RNA, and the second set has the T7 polymerase promoter sequence on the 3' primer to make the antisense RNA. PCR was performed in a 50 μ l reaction containing 36.5 μ l H₂O, 5 μ l 10xTT buffer (140 mM Ammonium Sulfate, 0.1 % gelatine, 0.6 M Tris-tricine pH 8.4), 5 μ l 25mM MgCl₂, 2 μ l 10 mM dNTP, 0.4 μ l Incyte clone 1722192 DNA, 0.5 μ l AmpliTaq (PE Applied Biosystems), and 0.3 μ l oligo1 (1 mg/ml) and 0.3 μ l oligo2 (1mg/ml)[to make the sense RNA], or 0.3 μ l oligo3 (1 mg/ml) and 0.3 μ l oligo4 (1mg/ml)[to make the antisense RNA]. The PCR reaction involved one cycle at 94°C for 2 min followed by 35 cycles at 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec. The two PCR reactions were loaded onto a 1.2 % agarose gel. The DNA band was excised from the gel, placed in a GenElute Agarose spin column (Supelco) and spun for 10 min at maximum speed. The eluted DNA was EtOH precipitated and resuspended in transcription buffer. The primer sequences for each nGPCR tested are listed below.

For nGPCR-11, the sense primers were:

GCGTAATACGACTCACTATAGGGAGACCGCGTGTCTGCTAGACTCTATTTCC
3'(LW1658) (SEQ ID NO: 159), and:

5' TGCCACACTGATGCAACTCC 3' (LW1661) (SEQ ID NO: 160). The antisense primers were:

position 13q32. Numerous investigations have found significant suggestion of linkage of schizophrenia to this region of chromosome 13q32. See, for example, Brzustowicz *et al.*, Am J Hum Genet 1999 Oct; 65(4): 1096-1103; Blouin *et al.*, Nat Genet 1998 Sep; 20(1): 70-3; Shaw *et al.*, Am J Med Genet. 1998 Sep 7; 81(5): 364-76; Lin *et al.*, Hum Genet 1997 Mar; 99(3): 417-20; Pulver *et al.*, Cold Spring Harb Symp Quant Biol 1996; 61:797-814.

Genes localized to chromosomal regions in linkage with schizophrenia are candidate genes for disease susceptibility. Genes in these regions with the potential to play a biochemical/functional role in the disease process (like G protein coupled receptors) have a high probability of being a disease-modifying locus. nGPCR-40 and -54, because of their chromosomal location, are attractive targets therefore for screening ligands useful in modulating cellular processes involved in schizophrenia.

EXAMPLE 13: CLONE DEPOSIT INFORMATION

In accordance with the Budapest Treaty, clones of the present invention have been deposited at the Agricultural Research Culture Collection (NRRL) International Depository Authority, 1815 N. University Street, Peoria, Illinois 61604, U.S.A. Accession numbers and deposit dates are provided below in Table 6.

Table 6: DEPOSIT INFORMATION

| Clone | Accession Number NRRL | Budapest Treaty Deposit Date |
|----------------------------|--------------------------|------------------------------|
| nGPCR-1 (SEQ ID NO:73) | B-30243 | 2000 Jan 18 |
| nGPCR -5 (SEQ ID NO: 75) | B-30244 | 2000 Jan 18 |
| nGPCR -16 (SEQ ID NO: 81) | B-30245 | 2000 Jan 18 |
| nGPCR -11 (SEQ ID NO: 79) | B-30258 | 2000 Feb 02 |
| nGPCR -17 (SEQ ID NO: 23) | B-30259 | 2000 Feb 03 |
| nGPCR -9 (SEQ ID NO: 77) | B-30262 | 2000 Feb 22 |
| nGPCR -58 (SEQ ID NO: 91) | B-30274 | 2000 March 23 |
| nGPCR -56 (SEQ ID NO: 89) | B-30288 | 2000 May 5 |
| nGPCR -3 (SEQ ID NO:185) | B-30290 | 2000 May 5 |
| nGPCR -54 (SEQ ID NO: 85) | B-30291 | 2000 May 5 |
| nGPCR -40 (SEQ ID NO: 83*) | B-30299N | 2000 June 02 |

* The clone deposited with NRLL Accession Number B30299N comprises a sequence identical to SEQ ID NO:83 but with the substitution of an "A" at nucleotide position 10.

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to a sequence of SEQ ID NO:192, and fragments thereof; said nucleic acid molecule encoding at least a portion of nGPCR-14, wherein the isolated nucleic acid comprises at least one or more nucleotides from one or more of the following regions of SEQ ID NO: 191: nucleotides 1 to 193 of SEQ ID NO: 191; nucleotides 612 to 644 of SEQ ID NO:191; nucleotides 697 to 706 of SEQ ID NO:191; nucleotides 1011 to 1049 of SEQ ID NO: 191; nucleotides 1051 to 1057 of SEQ ID NO:191; nucleotides 1090 to 1096 of SEQ ID NO:191; or nucleotides 1141 to 1642 of SEQ ID NO:191.
2. The isolated nucleic acid molecule of claim 1 comprising a sequence that encodes a polypeptide comprising a sequences of SEQ ID NO:192, and fragments thereof.
3. The isolated nucleic acid molecule of claim 1 comprising a sequence homologous to a sequence of SEQ ID NO:191, and fragments thereof.
4. The isolated nucleic acid molecule of claim 1 comprising a sequence of SEQ ID NO:191, and fragments thereof.
5. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is DNA.
6. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is RNA.
7. An expression vector comprising a nucleic acid molecule of any one of claims 1 to 5.
8. The expression vector of claim 7 wherein said nucleic acid molecule comprises a sequence of SEQ ID NO:191.
9. The expression vector of claim 7 wherein said vector is a plasmid.

10. The expression vector of claim 7 wherein said vector is a viral particle.
11. The expression vector of claim 10 wherein said vector is selected from the group consisting of adenoviruses, baculoviruses, parvoviruses, herpesviruses, poxviruses, adeno-
5 associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses.
12. The expression vector of claim 7 wherein said nucleic acid molecule is operably connected to a promoter selected from the group consisting of simian virus 40, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney
10 virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metallothionein.
13. A host cell transformed with an expression vector of claim 7.
15
14. The transformed host cell of claim 13 wherein said cell is a bacterial cell.
15. The transformed host cell of claim 14 wherein said bacterial cell is E. coli.
- 20 16. The transformed host cell of claim 13 wherein said cell is yeast.
17. The transformed host cell of claim 16 wherein said yeast is S. cerevisiae.
18. The transformed host cell of claim 13 wherein said cell is an insect cell.
25
19. The transformed host cell of claim 18 wherein said insect cell is S. frugiperda.
20. The transformed host cell of claim 13 wherein said cell is a mammalian cell.
- 30 21. The transformed host cell of claim 20 wherein mammalian cell is selected from the group consisting of chinese hamster ovary cells, HeLa cells, African green monkey kidney cells, human 293 cells, and murine 3T3 fibroblasts.

22. An isolated nucleic acid molecule comprising a nucleotide sequence complementary to at least a portion of a sequence of SEQ ID NO:191, said portion comprising at least 10 nucleotides, wherein said isolated nucleic acid comprises at least one or more nucleotides from one or more of the following regions of SEQ ID NO: 191: nucleotides 1 to 193 of SEQ
5 ID NO: 191; nucleotides 612 to 644 of SEQ ID NO:191; nucleotides 697 to 706 of SEQ ID NO:191; nucleotides 1011 to 1049 of SEQ ID NO: 191; nucleotides 1051 to 1057 of SEQ ID NO:191; nucleotides 1090 to 1096 of SEQ ID NO:191; or nucleotides 1141 to 1642 of SEQ ID NO:191.
23. The nucleic acid molecule of claim 22 wherein said molecule is an antisense oligonucleotide directed to a region of a sequence of SEQ ID NO:191.
24. The nucleic acid molecule of claim 23 wherein said oligonucleotide is directed to a regulatory region of a sequence of SEQ ID NO:191.
25. A composition comprising a nucleic acid molecule of any one of claims 1 to 5 or 22 and an acceptable carrier or diluent.
26. A composition comprising a recombinant expression vector of claim 7 and an
20 acceptable carrier or diluent.
27. A method of producing a polypeptide that comprises a sequence of SEQ ID NO: 192, and homologs and fragments thereof, said method comprising the steps of:
- a) introducing a recombinant expression vector of claim 7 into a
25 compatible host cell;
 - b) growing said host cell under conditions for expression of said polypeptide; and
 - c) recovering said polypeptide,
- wherein the polypeptide comprising a sequence of SEQ ID NO:192 comprises at least
30 one or more amino acid residues from one or more of the following regions of SEQ ID NO: 192: amino acid residues 1 to 42 of SEQ ID NO:192; amino acid residues 68 to 77 of SEQ ID NO:192; amino acid residues 185 to 197 of SEQ ID NO: 192; or amino acid residues 293 to 513 of SEQ ID NO: 192.

28. The method of claim 27 wherein said host cell is lysed and said polypeptide is recovered from the lysate of said host cell.

29. The method of claim 27 wherein said polypeptide is recovered by purifying the culture medium without lysing said host cell.

30. An isolated polypeptide encoded by a nucleic acid molecule of claim 1, wherein the polynucleotide comprises at least one or more amino acid residues from one or more of the following regions of SEQ ID NO: 192: amino acid residues 1 to 42 of SEQ ID NO:192; amino acid residues 68 to 77 of SEQ ID NO:192; amino acid residues 185 to 197 of SEQ ID NO: 192; or amino acid residues 293 to 513 of SEQ ID NO: 192.

31. The polypeptide of claim 30 wherein said polypeptide comprises a fragment of SEQ ID NO:192.

32. The polypeptide of claim 30 wherein said polypeptide comprises an amino acid sequence homologous to a sequence of SEQ ID NO:192.

33. The polypeptide of claim 30 wherein said sequence homologous to a sequence of SEQ ID NO:192 comprises at least one conservative amino acid substitution compared to the sequence of SEQ ID NO: 192.

34. The polypeptide of claim 30 wherein said polypeptide comprises a fragment of a polypeptide with a sequence of SEQ ID NO:192.

35. A composition comprising a polypeptide of claim 30 and an acceptable carrier or diluent.

36. An isolated antibody which binds to an epitope on a polypeptide of claim 30.

37. The antibody of claim 36 wherein said antibody is a monoclonal antibody.

38. A composition comprising an antibody of claim 36 and an acceptable carrier or diluent.

39. A method of inducing an immune response in a mammal against a polypeptide of claim 30 comprising administering to said mammal an amount of said polypeptide sufficient to induce said immune response.

5

40. A method for identifying a compound which binds nGPCR-14 comprising the steps of:

- a) contacting nGPCR-14 with a compound; and
- b) determining whether said compound binds nGPCR-14.

10

41. The method of claim 40 wherein the nGPCR-14 comprises an amino acid sequence of SEQ ID NO: 192.

42. The method of claim 40 wherein binding of said compound to nGPCR-14 is
15 determined by a protein binding assay.

43. The method of claim 40 wherein said protein binding assay is selected from the group consisting of a gel-shift assay, Western blot, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking,
20 interaction trap/two-hybrid analysis, southwestern analysis, and ELISA.

44. A compound identified by the method of claim 40.

45. A method for identifying a compound which binds a nucleic acid molecule encoding
25 nGPCR-14 comprising the steps of:

- a) contacting said nucleic acid molecule encoding nGPCR-14 with a compound; and
- b) determining whether said compound binds said nucleic acid molecule.

30 46. The method of claim 45 wherein binding is determined by a gel-shift assay.

47. A compound identified by the method of claim 45.

48. A method for identifying a compound which modulates the activity of nGPCR-14 comprising the steps of:

- a) contacting nGPCR-14 with a compound; and
- b) determining whether nGPCR-14 activity has been modulated.

5

49. The method of claim 48 wherein the nGPCR-14 comprises an amino acid sequence of SEQ ID NO: 192.

50. The method of claim 48 wherein said activity is neuropeptide binding.

10

51. The method of claim 48 wherein said activity is neuropeptide signaling.

52. A compound identified by the method of claim 48.

15 53. A method of identifying an animal homolog of nGPCR-14 comprising the steps:

- a) comparing the nucleic acid sequences of the animal with a sequence of SEQ ID NO: 191, and portions thereof, said portions being at least 10 nucleotides; and
- b) identifying nucleic acid sequences of the animal that are homologous to said sequence of SEQ ID NO: 191, and portions thereof.

20

54. The method of claim 53 wherein comparing the nucleic acid sequences of the animal with a sequence selected of SEQ ID NO: 191, and portions thereof, said portions being at least 10 nucleotides, is performed by DNA hybridization.

25 55. The method of claim 53 wherein comparing the nucleic acid sequences of the animal with a sequence selected of SEQ ID NO: 191, and portions thereof, said portions being at least 10 nucleotides, is performed by computer homology search.

56. A method of screening a human subject to diagnose a disorder affecting the brain or
30 genetic predisposition therefor, comprising the steps of:
(a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression, or biological activity of at least one nGPCR that is expressed in the brain, wherein the nGPCR comprises an amino acid

sequence of SEQ ID NO:192, and allelic variants thereof, and wherein the nucleic acid corresponds to a gene encoding the nGPCR; and

- (b) diagnosing the disorder or predisposition from the presence or absence of said mutation, wherein the presence of a mutation altering the amino acid sequence,
5 expression, or biological activity of the nGPCR in the nucleic acid correlates with an increased risk of developing the disorder.

10 57. A method according to claim 56, wherein the nGPCR is nGPCR-14 comprising an amino acid sequence set forth in SEQ ID NO:192 or an allelic variant thereof.

58. A method according to claim 56, wherein the assaying step comprises at least one procedure selected from the group consisting of:

- a) comparing nucleotide sequences from the human subject and reference sequences and determining a difference of at least a nucleotide or at least one codon between
15 the nucleotide sequences from the human subject that encodes an nGPCR-14 allele and an nGPCR-14 reference sequence;

(b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences;

- 20 (c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and

(d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from
25 one or more reference sequences.

59. A method according to claim 58 wherein the assaying step comprises: performing a polymerase chain reaction assay to amplify nucleic acid comprising nGPCR-14 coding sequence, and determining nucleotide sequence of the amplified nucleic acid.

30

60. A method of screening for an nGPCR-14 mental disorder genotype in a human patient, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including sequences corresponding to alleles of nGPCR-14; and

(b) detecting the presence of one or more mutations in the nGPCR-14 allele;

wherein the presence of a mutation in an nGPCR-40 allele or nGPCR-54 allele is indicative of a mental disorder genotype.

61. The method according to claim 59 wherein said biological sample is a cell sample.

62. The method according to claim 59 wherein said detecting the presence of a mutation comprises sequencing at least a portion of said nucleic acid, said portion comprising at least one codon of said nGPCR-14 alleles.

63. The method according to claim 59 wherein said nucleic acid is DNA.

64. The method according to claim 59 wherein said nucleic acid is RNA.

65. A kit for screening a human subject to diagnose a mental disorder or a genetic predisposition therefor, comprising, in association:

(a) an oligonucleotide useful as a probe for identifying polymorphisms in a human nGPCR-14 gene, the oligonucleotide comprising 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human nGPCR-14 gene sequence or nGPCR-14 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution; and

(b) a media packaged with the oligonucleotide, said media containing information for identifying polymorphisms that correlate with schizophrenia or a genetic predisposition therefor, the polymorphisms being identifiable using the oligonucleotide as a probe.

66. A method of identifying a nGPCR allelic variant that correlates with a mental disorder, comprising steps of:

(a) providing a biological sample comprising nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny;

(b) detecting in the nucleic acid the presence of one or more mutations in an nGPCR that is expressed in the brain, wherein the nGPCR comprises an amino acid sequence of SEQ ID NO:192, and allelic variants thereof, and wherein the nucleic acid includes sequence corresponding to the gene or genes encoding nGPCR;

wherein the one or more mutations detected indicates an allelic variant that correlates with a mental disorder.

10

67. A method according to claim 66 wherein the at least one nGPCR is nGPCR-14, or an allelic variant thereof.

68. A purified and isolated polynucleotide comprising a nucleotide sequence encoding a nGPCR-14 allelic variant identified according to claim 67.

15

69. A host cell transformed or transfected with a polynucleotide according to claim 68 or with a vector comprising the polynucleotide.

70. A purified polynucleotide comprising a nucleotide sequence encoding nGPCR-14 of a human with a mental disorder;

20

wherein said polynucleotide hybridizes to the complement of SEQ ID NO:191 under the following hybridization conditions:

(a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate and

25

(b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS; and

wherein the polynucleotide that encodes the nGPCR-14 amino acid sequence of the human differs from SEQ ID NO:192 by at least one residue and comprises at least one or more amino acid residues from one or more of the following regions of SEQ ID NO: 192: amino acid residues 1 to 42 of SEQ ID NO:192; amino acid residues 68 to 77 of SEQ ID NO:192; amino acid residues 185 to 197 of SEQ ID NO: 192; or amino acid residues 293 to 513 of SEQ ID NO: 192.

30

71. A vector comprising a polynucleotide according to claim 70.

72. A host cell that has been transformed or transfected with a polynucleotide according
5 to claim 70 and that expresses the nGPCR-14 protein encoded by the polynucleotide.

73. A host cell according to claim 72 that has been co-transfected with a polynucleotide
encoding the nGPCR-14 amino acid sequence set forth in SEQ ID NO:192 and that expresses
the nGPCR-14 protein having the amino acid sequence set forth in SEQ ID NO:192.

10

74. A method for identifying a modulator of biological activity of nGPCR-14 comprising
the steps of:

- a) contacting a cell according to claim 72 in the presence and in
the absence of a putative modulator compound;
- 15 b) measuring nGPCR-14 biological activity in the cell;
wherein decreased or increased nGPCR-14 biological activity in the presence versus
absence of the putative modulator is indicative of a modulator of biological activity.

75. A method to identify compounds useful for the treatment of a mental disorder, said
20 method comprising steps of:

- (a) contacting a composition comprising nGPCR-14 with a compound
suspected of binding nGPCR-14;
- (b) detecting binding between nGPCR-14 and the compound suspected
of binding nGPCR-14;
- 25 wherein compounds identified as binding nGPCR-14 are candidate compounds useful
for the treatment of a mental disorder.

76. A method for identifying a compound useful as a modulator of binding between
nGPCR-14 and a binding partner of nGPCR-14 comprising the steps of:

- 30 (a) contacting the binding partner and a composition comprising
nGPCR-14 in the presence and in the absence of a putative modulator compound;
- (b) detecting binding between the binding partner and nGPCR-14;
wherein decreased or increased binding between the binding partner and
nGPCR-14 in the presence of the putative modulator, as compared to binding in the absence

of the putative modulator is indicative a modulator compound useful for the treatment of schizophrenia.

77. A method according to claim 75 or 76 wherein the composition comprises a cell
5 expressing nGPCR-14 on its surface.

78. A method according to claim 77 wherein the composition comprises a cell transformed or transfected with a polynucleotide that encodes nGPCR-14.

10 79. A method of purifying a G protein from a sample containing said G protein comprising the steps of:

- a) contacting said sample with a polypeptide of claim 1 for a time sufficient to allow said G protein to form a complex with said polypeptide;
- b) isolating said complex from remaining components of said sample;
- 15 c) maintaining said complex under conditions which result in dissociation of said G protein from said polypeptide; and
- d) isolating said G protein from said polypeptide.

80. The method of claim 79 wherein said sample comprises an amino acid sequence of
20 SEQ ID NO:192.

81. The method of claim 79 wherein said polypeptide comprises an amino acid sequence homologous to a sequence of SEQ ID NO:192.

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 <212> PRT
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<400> 20

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          20           25           30
Asn Val Leu Ser Ala Val Cys Tyr Cys Ile Val Asn Lys Thr Phe Ser
          35           40           45
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Ala Lys Phe Ser Leu Arg Glu Asn Pro
65           70

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catggcactc acaaatttgc agaagggcag cccaaacatc caagtcttct tgatgaggta      180
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accaccagc cgcgaataa gcactatgaa gtagaggctg attaagtggg gtgtcactat      360
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 <212> PRT
 <213> Homo sapiens

<400> 22

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          20           25           30
Pro His Leu Ile Ser Leu Tyr Phe Ile Val Leu Ile Gly Gly Leu Val
          35           40           45
Gly Val Ile Ser Ile Leu Phe Leu Leu Val Lys Met Asn Thr Arg Ser
          50           55           60
Val Thr Thr Met Ala Val Ile Asn Leu Val Val Val His Ser Val Phe
65           70           75           80
Leu Leu Thr Val Pro Phe Arg Leu Thr Tyr Leu Ile Lys Lys Thr Trp

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85 90 95

Met Phe Gly Leu Pro Phe Cys Lys Phe Val Ser Ala Met Leu His Ile
100 105 110

His Met Tyr Leu Thr Val Pro Ile Leu Cys Gly Asp Pro Gly His Gln
115 120 125

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INTERNATIONAL SEARCH REPORT

Inte Application No
PCT/US 01/04641

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, EPO-Internal, MEDLINE, WPI Data, CHEM ABS Data, BIOSIS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | <p>DATABASE EMBL 'Online! EBI; AC008547, 4 August 1999 (1999-08-04) DOE JOINT GENOME INSTITUTE STANFORD HUMAN GENOME CENTRE: "Homo sapiens chromosome 5 clone CTC-502M5 complete sequence" XP002179737 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p style="text-align: center;">--- -/-</p> | 1-39 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

17 October 2001

Date of mailing of the international search report

07/11/2001

Name and mailing address of the ISA

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Authorized officer

Celler, J

INTERNATIONAL SEARCH REPORT

Inte: Application No

PCT/US 01/04641

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | <p>DATABASE EMBL 'Online! EBI; AI187401, 13 October 1998 (1998-10-13) STRAUSBERG R. : "Homo sapiens cDNA clone " XP002179738 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> | 1-39 |
| X | <p>DATABASE EMBL 'Online! EBI; AI768383, 30 June 1999 (1999-06-30) STRAUSBERG R.: "Homo sapiens cDNA clone " XP002179739 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> | 1-39 |
| X | <p>DATABASE EMBL 'Online! EBI; AI798227, 7 July 1999 (1999-07-07) STRAUSBERG R. : "Homo sapiens cDNA clone" XP002179740 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> | 1-39 |
| X | <p>DATABASE EMBL 'Online! EBI; AI140809, 28 September 1998 (1998-09-28) STRAUSBERG R.: "Homo sapiens cDNA clone" XP002179741 the whole document Also provided alignment with present SEQ. ID. NO.: 191 Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> | 1-39 |
| X | <p>DATABASE EMBL 'Online! EBI; BE044200, 17 June 2000 (2000-06-17) STRAUSBERG R. : "Homo sapiens cDNA clone" XP002179742 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> | 1-39 |
| X | <p>DATABASE EMBL 'Online! EBI; AA845448, 10 March 1998 (1998-03-10) STRAUSBERG R. : "Homo sapiens cDNA clone" XP002179743 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> <p>---</p> <p style="text-align: center;">-/--</p> | 1-39 |

INTERNATIONAL SEARCH REPORT

Inte Application No
PCT/US 01/04641

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | <p>DATABASE EMBL 'Online! EBI; AI051313, 13 July 1998 (1998-07-13) STRAUSBERG R.: "Homo sapiens cDNA clone" XP002179744 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> | 1-39 |
| X | <p>----- DATABASE EMBL 'Online! EBI; AW592243, 23 March 2000 (2000-03-23) STRAUSBERG R.: "Homo sapiens cDNA clone" XP002179745 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> | 1-39 |
| X | <p>----- DATABASE EMBL 'Online! EBI; AI470444, 17 March 1999 (1999-03-17) STRAUSBERG R.: "Homo sapiens cDNA clone" XP002179746 the whole document Also provided alignment with present SEQ. ID. NO.: 191</p> | 1-39 |
| X | <p>----- DATABASE SWALL 'Online! EBI; P47901, 1 February 1996 (1996-02-01) SUGIMOTO T. ET AL: "Vasopressin V1B receptor" XP002179747 the whole document Also provided alignment with present SEQ. ID. NO.: 192</p> | 1-39 |
| X | <p>----- DATABASE SWALL 'Online! EBI; Q9JLP2, 1 October 2000 (2000-10-01) O'CARROLL A.M. ET AL: "Arginine vasopressin tyoe 1B receptor" XP002179748 the whole document Also provided alignment with present SEQ. ID. NO.: 192</p> | 1-39 |
| X | <p>----- DATABASE SWALL 'Online! EBI; p70536, 1 November 1997 (1997-11-01) ROZEN F. ET AL: "Oxitocin receptor" XP002179749 the whole document</p> <p>-----</p> | 1-39 |

-/-

INTERNATIONAL SEARCH REPORT

Inter Application No

PCT/US 01/04641

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>DATABASE SWALL 'Online! EBI; Q9QW18, 1 May 2000 (2000-05-01) MOREL A. ET AL: "V1A arginine vasopresin receptor" XP002179750 the whole document Also provided alignment with present SEQ. ID. NO.: 192</p> | 1-39 |
| X | <p>DATABASE SWALL 'Online! EBI; Q62463, 15 July 1998 (1998-07-15) KIKUCHI S. ET AL: "Vasopressin V1A receptor" XP002179751 the whole document Also provided alignment with present SEQ. ID. NO.: 192</p> | 1-39 |
| E | <p>WO 01 36471 A (ARENA PHARMACEUTICALS INC ;CHEN RUOPING (US); DANG HUONG T (US); L) 25 May 2001 (2001-05-25) abstract SEQ. ID. NO.: 21</p> | 1-39 |
| E | <p>WO 01 36473 A (PARODI LUIS A ;LIND PETER (SE); SEJLITZ TORSTEN (SE); SCHELLIN KAT) 25 May 2001 (2001-05-25) abstract SEQ. ID. NO.: 15</p> | 1-39 |
| A | <p>WO 99 28470 A (GOODEARL ANDREW D J ;XIE MICHAEL (US); DISTEFANO PETER (US); GLUCK) 10 June 1999 (1999-06-10) the whole document</p> | 1-39 |
| A | <p>WO 98 46620 A (MILLENNIUM PHARM INC) 22 October 1998 (1998-10-22) the whole document</p> | 1-39 |
| A | <p>WO 99 63087 A (HODG MARTIN R ;GLUCKSMANN MARIA ALEXANDRA (US); MILLENNIUM PHARM I) 9 December 1999 (1999-12-09) the whole document</p> | 1-39 |

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-43,45-46,79-81(all partly),44,47-52,56-78

Present Claims 40-52,56-81 relate to products, compound and methods defined by reference to a desirable characteristics or property, namely polypeptides of nGPCR-14 with mutations, isolated specific antibodies thereto, compounds binding or modulating the activity of the molecules of the application, G-protein interacting with the polypeptides of the application, wherein the mutants, variants and alleles of nGPCR-14 are associated with a brain and/or mental disorder in humans or genetic predisposition thereto.

The claims cover products, compounds and methods having these characteristics or properties, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any such products, compounds and methods. Consequently the function which is prima facie assigned to the product encoded by SEQ. ID. NO.: 191 and/or having the sequence SEQ. ID. NO.: 192 and the variants thereof is not credible and has therefore been neglected for the purpose of this search.

There is no convincing evidence provided in the application to show that the putative gene characterised by the nucleotide sequence of SEQ. ID. NO.: 191, encoding putative protein nGPCR-14, characterised by the amino acid sequence of SEQ. ID. NO.: 192 is transcribed in vivo into mRNA, no evidence at all to show that the putative transcription is associated with the brain or neuronal tissues in any fashion. There is a complete lack of evidence to show that a polypeptide is translated from the transcript of said putative gene. Moreover the comparison of the amino acid sequence SEQ. ID. NO.: 192 with the sequences of GPCR with known functions revealed highest similarity with oxytocin receptor and vasopressin receptor. Even applying the structural similarities with said known receptors does not seem to indicate any particular or specific involvement of the putative nGPCR of the present application in functioning of the brain. Therefore there is no objective evidence to suggest that the function of nGPCR-14 is neither specifically associated with the brain, as claimed, nor any other neuronal tissues.

As a consequence of the lack of credible specific function a pure structure-based search has been conducted for Claims 1-39 in respect of SEQ. ID. NO.: 191 per se and SEQ. ID. NO. 192 per se. Claims 40-43 were searched only in so far as nGPCR-14 is defined by SEQ. ID. NO.:192 and/or encoded by polynucleotide of SEQ. ID. NO.: 191. Claims 45-46 were searched also in so far as nGPCR-14 is defined by SEQ. ID. NO.: 192 and/or encoded by polynucleotide of SEQ. ID. NO.: 191. Claims 48-51, 56-67 and 74-78 were not searched at all because, as outlined above, the putative receptor nGPCR-14 has no function assigned to it in a credible fashion. Claims 79-81 were searched only in so far as the "polypeptide of claim 1" 'step (a) of the method of claim 79' is characterised by SEQ. ID. NO. 192 and/or is encoded by a polypeptide of SEQ. ID. NO.: 191

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims 53-55 were fully searched.

Present Claims 44,47,52 and 68-73 relate to compounds defined by reference to a desirable characteristic or property. An attempt is made to define the compounds by reference to a result to be achieved, namely compounds found by methods of screening without providing any positive technical feature or examples of said compounds, which is indispensable at distinguishing the compounds for which protection is sought from all the compounds known in the art. Said claims simply recite the problem to be solved and completely fail to define any solution to said problem. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search was carried for Claims 44,47,52,68-73.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the polynucleotide characterised by the nucleotide sequence SEQ. ID. NO.: 191 and the putative polypeptide encoded thereby, characterised by the amino acid sequence of SEQ. ID. NO.: 192.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte

Application No

PCT/US 01/04641

| Patent document cited in search report | | Publication date | | Patent family member(s) | Publication date |
|---|---|---------------------|----|----------------------------|---------------------|
| WO 0136471 | A | 25-05-2001 | AU | 1769601 A | 30-05-2001 |
| | | | WO | 0136471 A2 | 25-05-2001 |
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| | | | AU | 1628299 A | 16-06-1999 |
| | | | EP | 1034268 A1 | 13-09-2000 |
| | | | WO | 9928470 A1 | 10-06-1999 |
| | | | US | 6093545 A | 25-07-2000 |
| WO 9846620 | A | 22-10-1998 | US | 5891720 A | 06-04-1999 |
| | | | AU | 6973698 A | 11-11-1998 |
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